UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

ULTRASONOGRAPHIC ASSESSMENT OF REPRODUCTIVE DISEASES IN GORILLAS AND OTHER CAPTIVE GREAT APES

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Ultrasonographic Assessment of Reproductive Diseases in Gorillas and other Captive Great Apes

Abstract

The present work focused on the analysis of ultrasound examinations from 29 male and female captive great apes performed since 1995 by the Leibniz-Institut für Zoo-und Wildtierforschung, IZW (Berlin, Germany), reproduction management group. The ultrasonographic appearance of the normal and abnormal reproductive tract was described.

Out of 22 female captive subjects, 18 were detected to have reproductive tract lesions. The altered ultrasound scans led to several suggested diagnoses, namely uterine leiomyomas, adenomyosis, nabothian cysts, functional ovarian cysts, paraovarian cyst, polycystic ovaries, endometrioma and hydrosalpinges in gorillas; cervical tumour and hydrosalpinx, in chimpanzees; and mostly functional ovarian cysts, in orangutans.

Two cases of pyosalpinx with involvement of the intestinal tract occurred, and the suspected causes were: acute pelvic inflammatory disease, in a gorilla, and diverticulitis in an orangutan.

Regarding the 7 male captive subjects, 3 gorillas were detected with testicular lesions. Suspicion of malignancy existed on the 3 cases, but only 1 was confirmed to be a Leydig cell tumour.

The collected data compared with the reproductive outcome of the subjects showed that (a) the absence of ultrasonographic alterations did not guarantee the production of offspring; (b) ultrasound examinations were efficient in identifying reproductively incompetent animals, since all the subjects whose suggested diagnosis was associated with a prognosis of “fertility most likely compromised” produced no offspring.

Key words: great apes, captivity, reproductive diseases, ultrasonographic diagnosis, fertility prognosis

Avaliação Ecográfica de Doenças Reprodutivas em Gorilas e outros Grandes Símios em Cativeiro

Resumo

O presente trabalho centrou-se na análise de ecografias ao trato reprodutivo de 29 grandes símios de ambos os sexos, obtidas desde 1995 pelo grupo de gestão de reprodução do Leibniz-Institut für Zoo-und Wildtierforschung, IZW (Berlim, Alemanha). Descreveu-se a aparência ecográfica do trato reprodutivo normal e anormal.

De entre 22 fêmeas cativas, detectaram-se lesões reprodutivas em 18 e as suas respectivas alterações ecográficas levaram a várias sugestões de diagnóstico, nomeadamente leiomiomas uterinos, adenomiose, quistos nabotianos, quistos ováricos funcionais, ovários poliquísticos, endometrioma e hidrosalpinges, em gorilas; tumor no cérvix e quisto paraovárico, em chimpanzés; e, maioritariamente, quistos ováricos funcionais, em orangotangos.

Encontraram-se 2 casos de piossalpinge com envolvimento do trato digestivo, sendo as causas sugeridas: doença inflamatória pélvica aguda, em uma gorila, e diverticulíte, em uma orangotango.

Relativamente aos 7 machos deste estudo, detectaram-se lesões testiculares em 3 gorilas. Houve suspeita de malignidade nos 3 casos, mas apenas em 1 se confirmou ser um tumor das células de Leydig.

A comparação dos dados obtidos com o desfecho reprodutivo dos pacientes mostrou que: (a) a ausência de lesões ecográficas não garantiu a produção de descendência; (b) os exames ecográficos foram eficientes na identificação dos animais reprodutivamente incompetentes, uma vez que todos os indivíduos cuja sugestão de diagnóstico estava associada a um prognóstico de “fertilidade muito provavelmente comprometida” não produziram descendência.

Palavras-chave: grandes símios, cativeiro, doenças reprodutivas, diagnóstico ecográfico, prognóstico de fertilidade

Training Report

The present work was conceived during and after a curricular internship in the IZW, Berlin, which lasted from February to August 2012.

The author recognizes that during this time a wide learning process took place, not only regarding the making of a scientific text but also valuable practical training by frequently assisting and collaborating in numerous projects in the course of several trips to local parks and foreign zoos. These trips involved working not only with great apes but with an array of zoo/wild animals like eurasian lynx, bears, wild cattle, naked mole rats, guinea pigs, roe deer, hares and elephants.

Generally speaking, this training allowed the dissertation's author to gain experience mostly in the reproductive management area, working with Prof. Dr. Thomas Hildebrand and Dr. Frank Göritz, namely handling the anaesthetic concerns (formulation of immobilization drugs doses, dart preparing and shooting, emergency drugs, monitoring vital stats, intubating, oxygen bottle preparation, IV catheterization, etc), performing and interpreting diagnostic imaging exams of the reproductive tract (ultrasonography, endoscopy) and finally assisting in the most relevant reproductive procedures like semen collection/evaluation and artificial insemination.

The author also participated in a Great Ape Health Meeting held at the London Zoo organized by the Zoological Society of London on May 2012.

Regarding the area of internal medicine, the author had the opportunity to perform clinical tasks during a few clinical cases of non-reproductive concern, thanks to joining the Lynx Project with Dr. Johanna Painer and Dr. Frank Göritz veterinary services to animal protection organizations like Vier Pfoten and others. In these trips the author learned how to perceive the normal and/or abnormal behaviour of the species, to gather the clinical data relevant for diagnosis before and after sedation, to select the appropriate tests to be performed, to prescribe the adequate medication and monitor their clinical improvement.

Finally, the author also joined other IZW internal projects, which included assisting in CT scans, helping on surgeries, inducing and assisting in the anaesthesia in the surgery room (IZW and Niederfinow), and providing the post-surgical care of the animals mentioned above.

In sum, through all the tasks supervised by extremely patient and explanatory staff members, the author managed to accomplish the goal of acquiring skills and competences in the area of interest, which will hopefully later allow for a fully based autonomy.

General Index

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The figures with no source identification are from IZW reproduction management group

Abbreviation Index

AI	Artificial insemination
bpm	beats per minute
CA125	Cancer antigen 125
CL	Corpus luteum
CT	Computed tomography
FSH	Follicle stimulating hormone
GIT	Gastrointestinal tract
GnRH	Gonadotropin releasing Hormone
IM	Intramuscular
IUCN	International Union for Conservation of Nature
IV	Intravenous
IZW	Leibniz Institut für Zoo- und Wildtierforschung
LH	Luteinizing hormone
MRI	Magnetic ressonance imaging
NCBI	National Center for Biotechnology Information
NHP	Non-human primate
PCOS	Polycystic ovarian syndrome
PID	Pelvic inflammatory disease
PLAP	Placental alkaline phosphatase
PO	Per os
TAUS	Transabdominal ultrasonography
TOA	Tubo-ovarian abscess
TRUS	Transrectal ultrasonography
TVUS	Transvaginal ultrasonography
US	Ultrasound
WHO	World Health Organization
TUI	Tomographic ultrasound imaging

1. Introduction

1.1. Great apes conservation context

The taxonomic family *Hominidae* includes the genus *Homo, Pan, Pongo* and *Gorilla* (National Center for Biotechnology Information [NCBI], 2012). The common term “great apes” can be used to refer to the entire taxonomic family or it can exclude humans (Groves, 2005). In this text the great apes only included the chimpanzee, bonobo, orangutan and gorilla.

Nowadays, all the great apes face serious threats in the wild due to habitat loss, climate change, infectious disease and poaching for both meat and the live pet trade. Table 1 shows the population estimate for great apes in the wild by the International Union for Conservation of Nature, IUCN, and also the extinction risk category to which they currently belong (IUCN, 2012). Although the numbers show a worrying situation for all the great apes, special attention is necessary to the Sumatran orangutan, Western gorilla (particularly the Cross River gorilla) and Mountain gorilla, which are classified as critically Endangered. These alarming data highlight the importance of a reproductive program in captivity, in order to maintain, as a last resource, the zoo and park animals as a conservation reserve for further repopulation of the wild.

Table 1. Estimated numbers of wild great ape populations (IUCN, 2012).

Species	Estimated individuals	IUCN redlist category
Chimpanzee (*Pan troglodytes*)	172,700 to 299,700 (*P. t. verus; P. t. ellioti; P. t. troglodytes; P. t. schweinfurthii*)	Endangered
Bonobo (*Pan paniscus*)	33,400	Endangered
Orangutan *(*Pongo *spp.)*	7,300 Sumatran orangutans(*P. Abelii*)	Critically Endangered
	45,000 to 69,000Bornean orangutans(*P. Pygmaeus*)	Endangered
Gorilla	95,000 Western gorillas (*G. gorilla. gorilla*; *G. gorilla diehli*)	Critically Endangered
	<17,000 Eastern gorillas(*G. beringei ssp. graueri*)	Endangered
	680 Mountain gorillas(*G. beringei beringei*)	Critically Endangered

Studies and comparisons between captive and wild great apes are beneficial to the management of both populations. However, when studying gorillas it should be taken into consideration that almost all gorillas found in captivity are western lowland gorillas while the majority of behavioural studies of free-ranging gorillas are from the mountain gorilla subspecies (Czekala & Robbins, 2001).

Presently, the captive great apes population in Zoos and Parks worldwide is around 750 gorillas, more than 1000 chimpanzees (Belfast zoo, 2012), nearly 900 orangutans (Great Apes Film Initiative [GAFI], 2012) and approximately 220 bonobos (Jacksonville zoo, 2012).

The reproductive management and conservation of captive great apes, as with most Zoo animals, is complex and requires computerised genetic planning, expensive and stressful translocations and there are no guarantees of reproductive compatibility, as animals can refuse to copulate due to sexual preferences (Pukazhenthi & Wildt, 2003). The implementation of reproductive technologies can potentially improve the offspring production in captive wildlife by combining assisted breeding and non-invasive methods of reproductive assessment (Pukazhenthi & Wildt, 2003). Since the reproductive capacity is strongly dependent on the health of the internal genital tract, it is important to detect pathologic alterations that may compromise reproductive performance before establishing breeding groups (Loskutoff, Kraemer, Raphael, Huntress & Wildt, 1991).

The diagnosis of the relevant reproductive disorders can be achieved through ultrasound examinations. Additionally, ultrasonography can also help to examine the influence of hormones released during different phases of the cycle on each genital tract structure providing insights into the reproductive processes, and in many cases, monitoring the effect of the applied treatments. Ultrasonography may also detect changes in size and structure of the testes and accessory glands characteristic of sexual maturity.

1.2. Objectives and Structure of the dissertation

The aim of this study was primarily to further the ultrasonographic knowledge of the captive great ape's normal and abnormal internal genital tract through a compilation of US images and suggested diagnoses. Furthermore, it was investigated if the suggested diagnosis of the collected data were related to reproductive performance outcome.

This text was divided in four chapters: introduction, materials & methods, results & discussion, and final considerations.

In the Introduction chapter, the published literature on the reproductive physiology and anatomy of great apes was reviewed to provide a knowledge foundation for the present topic.

Materials & Methods described the characteristics of the studied subjects and the equipment and methods used by the IZW reproduction management group.

The Results & Discussion chapter was structured so that the normal ultrasonographic appearance of the reproductive tract is described in detail for male and female great apes

before analysing and discussing the pathologies. The encountered lesions were grouped according to their reproductive tract location (uterus, adnexa, testicule) and ultrasonographic characteristics (cystic, solid, complex). For every lesion group, differential diagnoses were presented and a suggested diagnosis was discussed based on the imagiologic experience of the author's supervisors, anamnesis, and on the published literature on the matter. The outcome and prognosis for each case was also included. Due to scarce information on great apes ultrasonography, the human-model as well as the non-human primate model were used rationally throughout the discussion points. On the last part of the Results & Discussion chapter, a small descriptive statistic study of the results was established and a reflection about the accuracy of ultrasound diagnostics and fertility prognostics in great apes was presented. In the end, general considerations were made resuming the work.

1.3. Great ape reproductive biology

It is fundamental to understand the reproductive physiology and anatomy of the great apes in order to interpret the diagnostic imaging obtained via ultrasound and be able to contribute to their clinical and reproductive management successfully.

The majority of the data in this topic are based on the works of Wislocki (1932), Collins (1981), Kingsley (1988), and Dixson (2012), and therefore constant referencing to these sources was avoided by mentioning these authors only in the dissertation titles that feature them. Information from other sources is referenced throughout the text.

1.3.1. Female Reproductive Physiology (Collins, 1981)

The menstrual cycle of the female great ape is characterized by the occurrence of menses, being readily visible in chimpanzees and occult in the urine of orangutan and gorillas.

Like the human female, the orangutan lacks any externally quantifiable labial or sexual swelling, but the gorilla, bonobo and chimpanzee display this type of swelling cyclically, and this occurrence was used for the terminology of the cycle's phases, as presented in Fig 1.

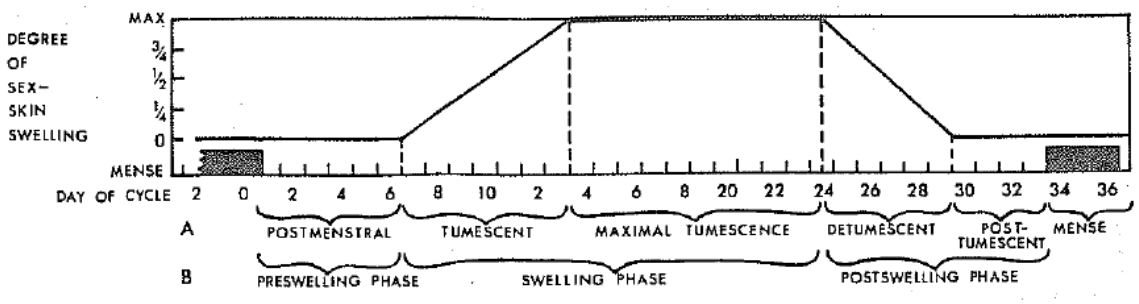

Fig 1. The phases of the chimpanzee reproductive cycle, showing alternative terminologies (Graham, 1970).

The female chimpanzee goes through changes in size and turgidity of the skin surrounding the genitalia providing valuable indication of the progress of the reproductive cycle. The maximal size of the sexual swelling varies from individual to individual and, with varying circumstances, within the same individual; reaching the vagina and the anus up to a volume of 1.4 dm^3 (Elder & Yerkes, 1936).

Female gorillas, on the other hand, when nulliparous, display only swelling of the labia, and multiparas have very subtle outward signs of ovulation. Wrinkling/smoothness of the labia with exposure of the mucosa and urogenital cleft are visual clues of their sexual state for the male.

Furthermore, there are no outward signs of pregnancy in these animals, so hormonal analysis can add much to basic observational data. (Czekala & Robbins, 2001)

Urinary ovarian steroid excretion and circulating hormones have been studied in female great apes in relation to labial swelling, and the comparative endocrinology with the human cycle exemplified in Fig 2.

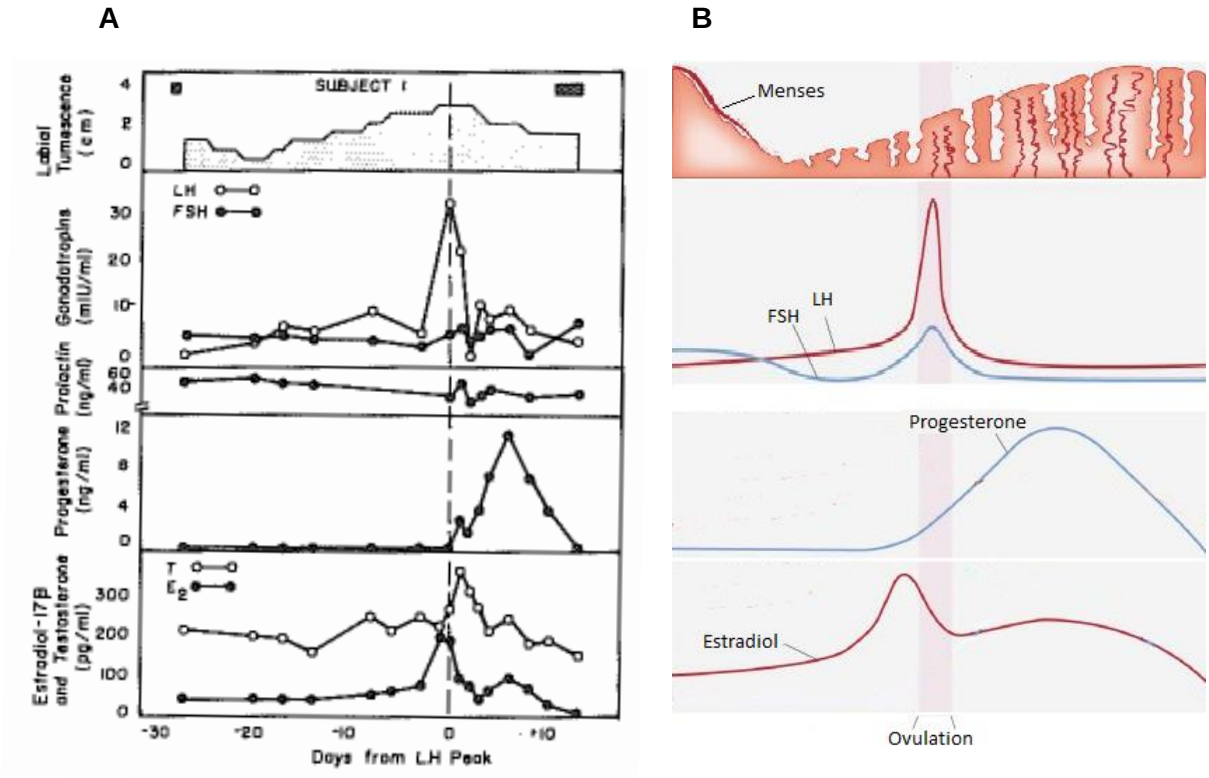

Fig 2. (A) Plasma LH, FSH, prolactin, estradiol, and testosterone and labial tumescence during the menstrual cycle of a female lowland gorilla, data are normalized to the day of the LH peak. Black bars indicate days of menstruation; T= testosterone; E2 = estradiol (Nadler, Graham, Collins & Gould, 1979); (B) morphology of the endometrium, FSH, LH, pogesterone and estradiol variations during the human menstrual cycle, data are normalized to estimated ovulation days (up until 24-36 h of the LH surge) - adapted from The Georgia Highlands College Library, [highlands.EDU], 2013).

Based on Fig 2, it can be perceived that there are a great number of similarities between the menstrual cycle of great apes and women (contrarily to the rhesus monkey's cycle), including: a midcycle estradiol peak that occurs one to 4 days previous to the LH peak, a pronounced midluteal elevation in estradiol (thought to be connected to luteolysis - approximately 7-10 days after LH peak) and relatively high concentrations of progesterone during the luteal phase.

Also, Fig 2 shows that the gonadotropin changes are correlated with the changes in labial swelling: labial swelling is minimal at menses, rises after six to 16 days, and reaches its maximum at 9 to 25 days after menses (usually on the day before the LH peak and, in association with estrogen's midcycle elevation). Detumescence occurs 0 to 3 days after LH peak, when estrogen is decreasing and progesterone is increasing. Morphology of the endometrium during the cycle, as shown in Fig 2 B, for humans, will be discussed later in this dissertation.

A comparative study of urinary reproductive hormones in great apes achieved by Shimizu et al. (2003) corroborated the previous knowledge that the pattern of gonadal steroids during the menstrual cycle in great apes more closely resembles that of human's than the long-tailed macaque. Their studies also showcased newly found differences between the taxa including: a) the urinary concentration of FSH whose pattern was similar in all great apes (with a marked mid-cycle rise and an early follicular phase rise) except in the gorilla (3 peaks during the menstrual cycle), and b) lower concentration of progesterone during the luteal phase in the orangutan when compared to the other great apes. The extra FSH peak in gorillas was an even earlier follicular phase rise thought to be related to the priming of the ovarian follicles or to follicle selection in the subsequent cycle (Dahl, Czekala, Lim & Hsueh, 1987).

Data about the menstrual cycle is extremely important for predicting ovulation time. In fact, time of ovulation is one of the most physiological significant parameters of the menstrual cycle, yet it is one of the most difficult events to precisely time.

In almost all species, including great apes, the evidence concerning time of ovulation is usually indirect, being based upon assumptions on the time interval between pre- or post-ovulatory physical events linked to hormonal changes. Thus, according to Gould (1982) detection of ovulation in great apes includes vague and subjective methods such as: measuring time relative to cyclic events as menstruation, perineal swelling, body temperature, viscosity of cervical mucus, and behaviour (copulation, masturbation, aggressivity); and objective methods, like observation of ovarian morphology using laparoscopy or ultrasonography and the practical but indirect test of hemagglutination inhibition of LH, for detecting the LH surge.

The time-scale of reproductive development of apes more closely approaches that of humans than does that of other primates that have been studied (Graham, 1970).

Adolescence, which occurs within the first years of life in monkeys, does not occur until 6-10 years of age among the apes. Once initiated, reproductive cyclicity in great apes persists into the fifth decade, as in women, culminating in menopause (Margulis, Atsalis, Bellem & Wielebnowski, 2007).

According to the literature, these reproductive parameters vary in the wild and captivity. Inconveniently, data about these topics is based on several studies that are usually concentrated in one of the conditions and/or don't make a comparison of all the parameters in both situations. Thus, the author compiled in Table 2 the results of these studies and referenced whether the study was in the wild or captive populations.

Average neonatal weight was included in Table 2 to stress the differences between human and great ape newborns. According to Leutenegger's (1973) studies, maternal and fetal (birth) weights are in allometric relationship with a correlation coefficient of 0.98; and since the constant of allometry is less than 1 (negative allometry), it indicates that primates of small adult body size have relatively larger newborns than primates of larger adult body size. Newborn gorillas, however, are exceptionally small, whereas human newborns are strikingly larger than expected on the basis of the regression.

The newborn weight and head size are important factors in reproduction, particularly in obstetrics, as will be discussed further in 1.3.3.6., together with the great ape pelvic anatomy. Despite small neonatal size, gorillas grow faster than the other great apes, including humans, achieving a larger adult size even though they mature earlier. Species with slow life histories generally have relatively later ages at weaning and longer interbirth intervals.

For mammals, great apes, and especially orangutans, wean their offspring relatively late (around 6–8 years old) and have long interbirth intervals (7–9 years). This can be a reproductive disadvantage for endangered species, and therefore, reproductive success is of utmost importance for their conservation (Beck, Rodrigues & Unwin, 2007).

Table 2. Reproductive parameters of great apes and humans

Species	Average Neonatal weight (kg)	Age at weaning (years)	Menarche (years)	Sexual Maturity (years)		Gestation length (days)	Interbirth Interval (years)		Cycle length (days)
				M (1st breed)	F (1st birth)		If infant survived	If infant died	
Gorilla b. beringei	2.2-2.6 *	3-4	7-7.5	15	10.1	256	3.91	1	28
Gorilla b. graueri	2.2-2.6 *	-	-	15	10.6	254	4.58	2.17	33
Gorilla g. gorilla	2.2-2.6 *	4	6.5-8.6	6-9.5	6-9.7	257	4.25	1.54	32-33
Pan troglodytes	1.78 †	4-5	10-11	12-13	14.3	228	5.4-6	-	36
Pan paniscus	1.162 ‡	-	-	-	8-15 Φ	244	6.25		32-36 [a]
Pongo spp	1.2-2.2 ¶	4,5	6-8	10 ***	15	240	8.05	-	28
Homo sapiens	2.5-3.3 §	2.8	12-14 **	-	19.5	279 ¥¥	1.25 $	2.25$	28.7^

All unmarked data was obtained from tables included in Groves & Meder (2001) and Robson & Wood (2008).
Data about *G.beringei*, *G. graueri*, *Pan troglodytes* and *Pongo spp* are from studies in wild populations; Data about *G.gorilla* is from captivity.
* – Coffey & Pook, 1974.
† – Fontaine, 2007.
‡ – Hill, 1968.
¶ – Bond, 1979.
§ – Peller, 1940.
** – Ten-Bosch, 1969.
Φ – Lathouwers & Elsaker, 2005.
¥¥ – Karn & Penrose, 1951.
[a] – Bolster &Savage-Rumbaugh, 1989.
*** – Wich et al., 2004.
^ 4154 – Gunn, Jenkin & Gunn, 1937.
$ 9519 – Saxton & Serwadda, 1969.

1.3.2. Male reproductive physiology (Kingsley, 1988)

Sexual maturity in mammals can be defined as the time of fertile sperm production and it is reached during the later stages of pubertal development.

However, in male great apes the onset of fertility is generally more difficult to assess than for females, as males do not exhibit well-defined physiological events that mark maturity such as the onset of menarche.

Because of this difficulty in recognizing when males initially become fertile, the classification of males into age categories - juvenile (J), adolescent (YA), adult (A) - is typically based on somewhat arbitrary established time-frames, namely for gorillas: (J) 3-6, (YA) 6-12, (A)>12 years old. Another measure of the maturity process is to determine when males go through hormonal puberty in relation to outward signs of reproductive behaviour and the development of secondary sexual characteristics.

In gorillas and orangutans the development of secondary sex characteristics marks the end of puberty and entrance into adulthood: silver back gorillas have a proeminent sagittal crest, emit a pungent odour and are larger comparing to adolescent black backs; flanged orangutans have pads of facial fat and muscle, a throat pouch, thicker and longer hair and are also larger than non-flanged males.

Although a fully developed adult male can only be considered after these physical changes, fertility can already occur in the adolescent phase, as there are reports of black backs copulating with adult females and, by paternity analysis, potentially siring infants (Bradley et al., 2005); and non-flanged orangutans being proven by paternity analysis to sire offspring in a group with flanged males (Utami, Goossens, Bruford, De Ruiter & van Hooff, 2002).

While the gorilla and the chimpanzee go through adolescence/puberty continuously, during a period of more or less 5 years (starting roughly around 6 years of age), the orangutan male can be fixed in the adolescent stage (non-flanged) for many years (20+), since the development into fully adult may be repressed for as long as the individual lives near a fully grown adult male. However, once separation from a flanged neighbour takes place, the development of secondary sexual characteristics occurs rapidly, usually within one year.

The changes in the concentration of urinary testosterone in the male gorilla and orangutan are similar to those of human and chimpanzees: Juvenile<Adolescent<Adults, with means ranging from 0.21 ng/mg NaCl in juveniles to 2.07 ng/mg NaCl in fully developed adults.

The early stages of development of secondary sex characteristics in the male orangutan are thought to be triggered by an increase of testosterone and estrogen excretion. Moreover, recently, low testosterone levels have been correlated with delayed development in male orangutans (Thompson, Zhou & Knott, 2012).

1.3.3. Comparative anatomy of the female reproductive tract (Wislocki, 1932)

The great apes are physiologically and anatomically closely related to human beings.

One of the biggest macroscopic differences, especially in Gorillas, is the attachment of the internal organs to the peritoneum by physiological fibrosis, often misdiagnosed as peritonitis, which makes laparoscopic examinations nearly impossible (Wildt, Chakraborty, Cambre, Howard & Bush, 1982).

In common with human females and almost all other primate species, great apes possess a simple unilocular uterus (uterus simplex) located within the pelvis, and two oviducts (or fallopian tubes), which lie between the uterus and the ovaries.

Contrary to other primates, great apes share with human females a lack of superior mesosalpinx and ovarian bursa, as well as a cervix of relatively moderate size (Fig3).

Regarding the external genitalia, great apes like the gorilla and orangutan have a vulva and clitoris of smaller dimensions than the chimpanzee and the human.

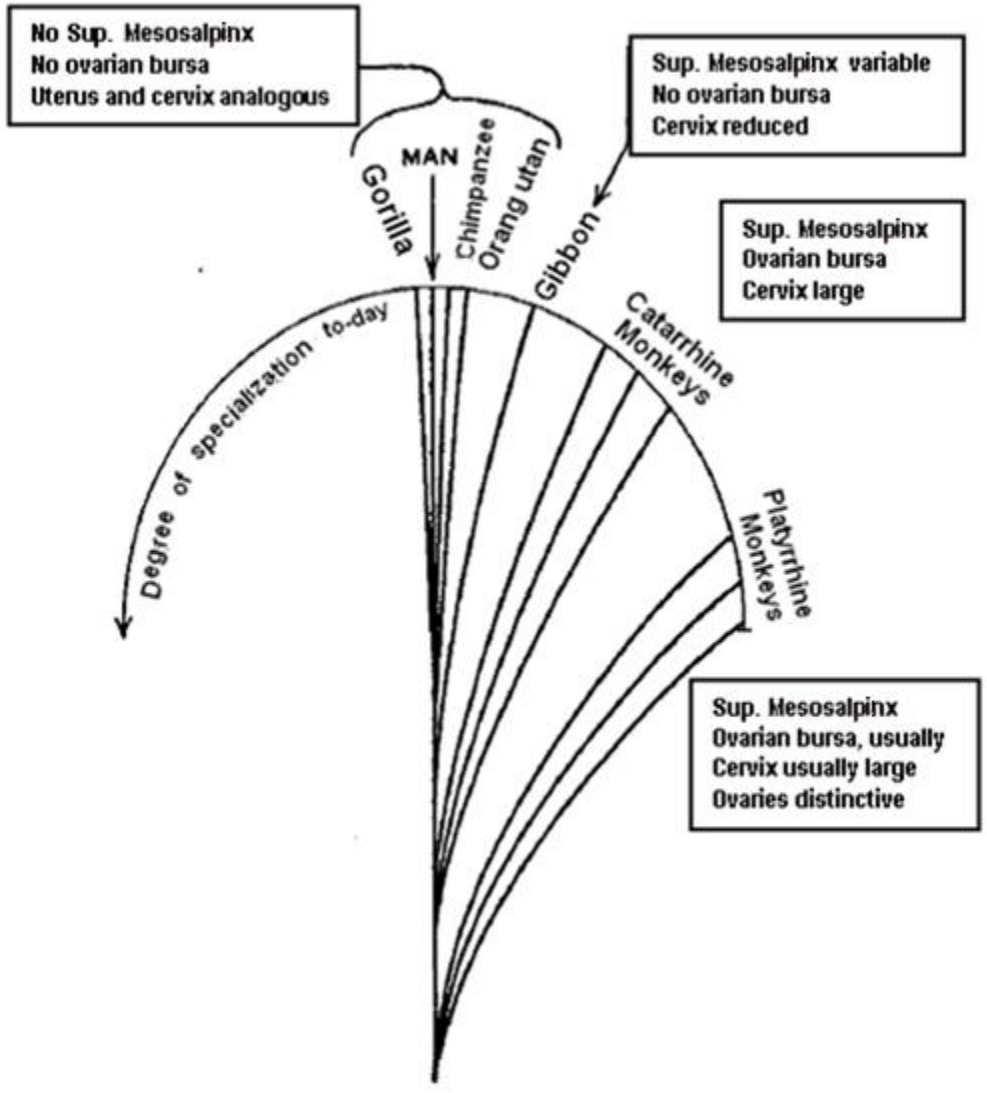

Fig 3. The general arrangement of the internal genitalia of *Pan, Pongo* and *Gorilla* is similar to that in *Homo* by Wislocki (1932).

In all apes, the unilocular uterus is situated dorsal to the bladder, and a rather straight cervical canal communicates to the exterior via a dilatable vagina, which is lined with a moderately rugose membrane. The internal genital tract organs and its relations to pelvic structures are depicted in Fig 4 and are described from 1.3.3.1 to 1.3.3.5.

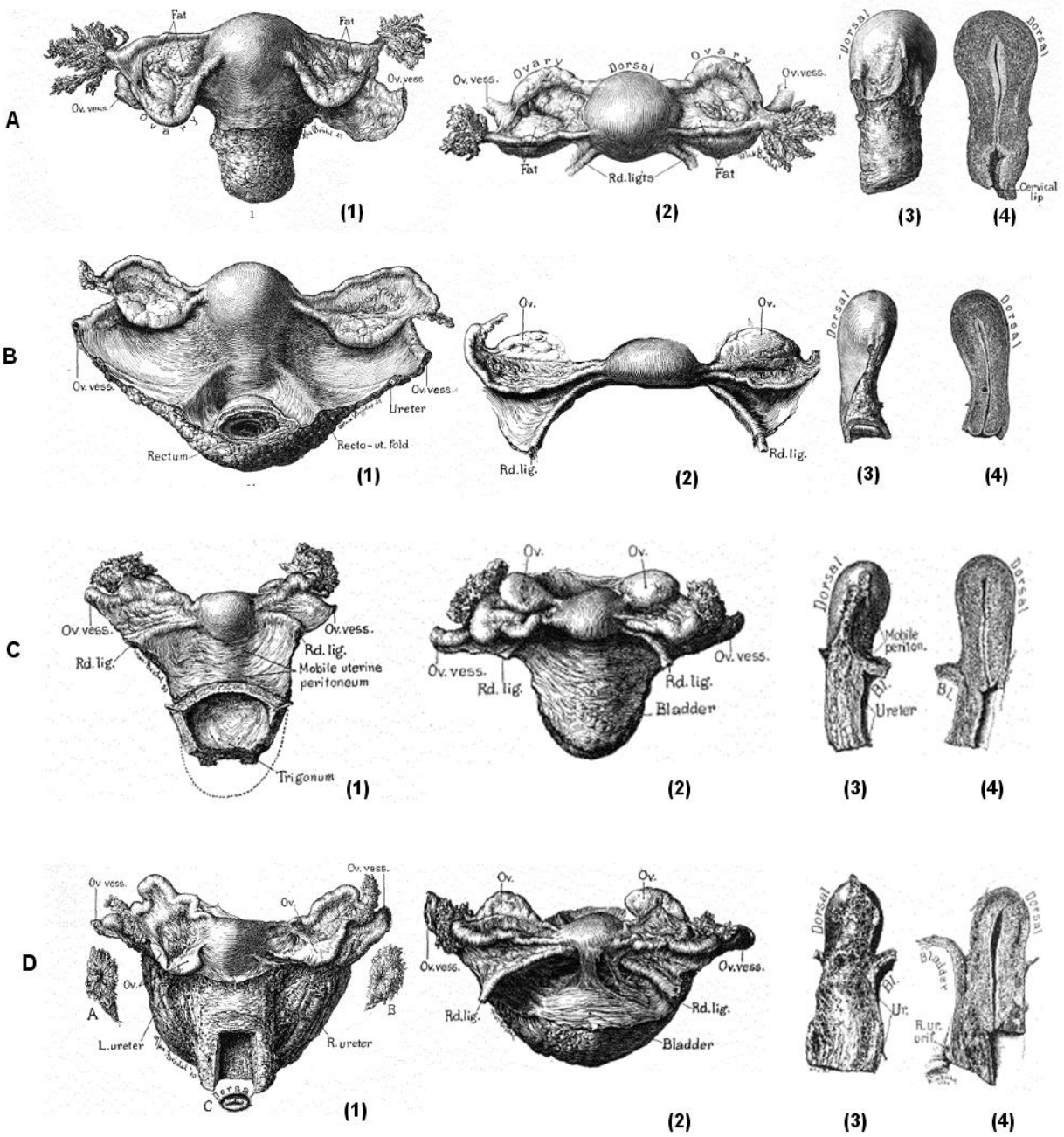

Fig4. Anatomy of adult uterus and adnexa of (A) gorilla, (B) human, (C) orangutan, (D) chimpanzee, in dorsal view (1), fundus view (2), lateral view (3) and midsagital plane (4); adapted from Wislocki (1932).

1.3.3.1. Ovaries

The ovaries of *Pan* and *Gorilla* have recorded dimensions ranging, in cm, from 1.5 x 0.5 x 0.2 to 4.5 x 2.5 x 2.0 and are within the accepted normal range for *Homo*.

1.3.3.2. Oviducts

It appears that the oviducts are more flexed in *Pan* than in *Homo* and are approximately 7 cm long. In Gorilla the oviducts measure 4.5 – 4.7 cm.

1.3.3.3. Uterus

The published literature on great apes suggests that the uterus of *Gorilla* is larger than that of *Homo*, that the uterus of *Pan* is approximately the same size as *Homo,* and that *Pongo* possesses a uterus considerably smaller than the other species. The increased size of the gorilla uterus has been attributed to extreme development of all the muscular elements and results in a reduction of the vesico-uterine and recto-uterine pockets (Douglas pouch). In both *Pan* and *Gorilla* the long axis of the uterus barely deviates from that of the vagina and differs from *Homo* in this regard. However, there are reports describing gorillas with uteri in anteverted and retroverted positions (Ball, Lynch, Olsen, Dumonceux & Burton, 2000).

The uterus of *Gorilla* is relatively exposed in comparison to that of *Pongo* and *Pan* that are deeper in the pelvis. In chimpanzees it is a frequent observation that the cervical canal is eccentrically placed within the ectocervix when visualized with the aid of a vaginal speculum. The endocervical mucosa varies in its extent, and the junction with the ectocervical mucosa may or may not be visible.

1.3.3.4. Vagina

The vagina of an adult female chimpanzee is approximately 12.5 cm long, and increases in diameter in the posterior direction. Dorsal and ventral fornices are present and well-developed, the latter being larger. The vagina does not open directly to the exterior in *Pan*, but via a vestibule formed by the sexual swelling that surrounds and involves the perineal area. The vagina of *Pongo* and *Gorilla* are smaller in all dimensions than that of *Pan*, but the general anatomy is similar.

1.3.3.5. Urethra

The urethra opens ventrally into the vaginal-vestibular canal approximately at the junction of these structures. Thus, it can only be visualized by means of a speculum.

1.3.3.6. Anatomic considerations regarding birth

As mentioned previously, the pelvic anatomy of the apes as well as the head size and weight of the newborn are important factors in their reproduction. Fig 5, shows that great apes have a relatively spacious pelvic inlet comparing to the monkeys and gibbon, whose dimensions of the infant cranium are only slightly smaller than the dimensions of the mother‘s pelvis. In humans, the infant cranium is actually longer than the anterior-posterior dimension of the pelvic inlet, requiring the head to enter the inlet facing sideways.

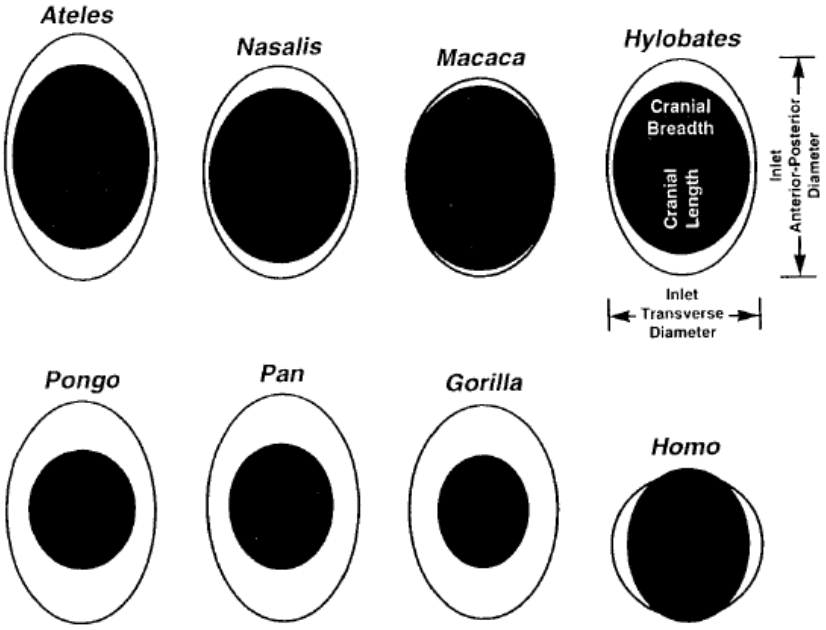

Fig 5. Size of pelvic inlet in relation to size of the newborn‘s head in several primates (Rosenberg & Trevathan, 2003).

As one would expect from examining these drawings, birth is reported to be difficult for the smaller-bodied primates (monkeys and gibbons), as well as for humans, but somewhat easier for the larger bodied great apes. Thus, New World monkeys and humans are especially susceptible to dystocias, because of the increased fetal size relative to maternal size. Dystocia, however, is defined as difficult labor and delivery due to many causes (maternal pelvic abnormalities and pathologies, twin pregnancy, etc) not only maternal-fetal disproportion and naturally, it has been reported in pro-simians, including great apes (Abee, Mansfield, Tardif & Morris, 2012). The early recognition and treatment of dystocia is critical to minimize possible life-threatening complications to the fetus and/or dam. In this regard, ultrasonography is a valuable tool for the examination and assessment of the fetus health during a dystocic delivery (Abee et al., 2012).

1.3.4. Comparative anatomy of the male Reproductive Tract (Dixson, 2012)

There are many basic similarities between man and the great apes concerning both the anatomy and the physiology of the male reproductive system. Descended testes with adjoining epididymides allow for sperm production, maturation and storage.

A vas deferens connects these structures to the urinary tract and allows for additional sperm storage. Accessory glands, notably the single prostate gland and paired seminal vesicles, provide additional ingredients to the fluid medium in which sperm is transported. These secretions mix together in the proximal portion of the urethra during ejaculation. A small amount of the secretion from the bulbo-urethral glands (Cowper's glands), released into the distal urethra, in advance of the bulk of the ejaculate, may help neutralize the acid environment of the urethra. External genitalia of quite differing size allow for variable vaginal penetration during copulation in several species. A gross view of the male urogenital tract is represented in Fig 6.

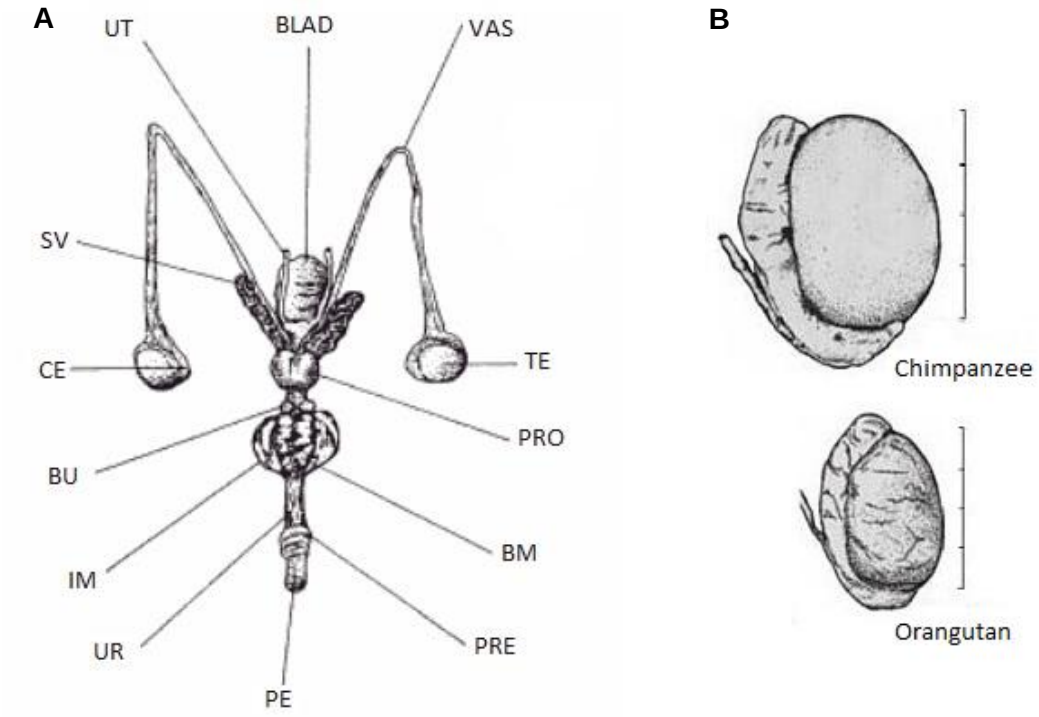

Fig. 6. (A) Schematic dissection of the reproductive tract of an adult orangutan (*Pongo pymaeus*) to show the major organs (Dixson, 2012). Blad = bladder, BM = bulbocavernosus muscles, BU = bulbo urethral gland, CE = cauda epididymis, IM = ischiocavernosus muscle, PE = penis, Pre = prepuce, Pro = prostate, SV = seminal vesicle, TE = testis, UR = urethra, VAS = vas deferens; (B) morphology and relative testis size of a chimpanzee and an orangutan (Dixson, 2012).

Information on the male great ape reproductive tract is very limited. Dixson (2012) compiled

the most relevant anatomic aspects of the male primate internal and external genital tract based on the works of Hill (1946) and Graham (1970) for chimpanzees and Oelrich (1978) and Graham (1988) for gorillas and orangutans.

1.3.4.1. Testes

The chimpanzee testis is roughly twice the weight of a human testis, while those of gorilla and orangutan are roughly 30% less. In addition, the testicular seminiferous epithelia are thick in the chimpanzee and orangutan but thin in the gorilla, whose interstitial tissue is abundant in Leydig cells. Not surprisingly, the sperm concentration in a typical chimpanzee ejaculate is many times greater than that seen in the gorilla (Fujii-Hanamoto, Matsubayashi, Nakano, Kusunoki & Enomoto, 2011)

Comparative studies of genital anatomy in male primates show that the differences in testis weight and size in great apes are related to their intrinsic mating systems. Male gorillas and orangutans copulate infrequently, and when a female comes into estrus she normally mates with only one male. However, in the chimpanzee, several males mate frequently with the oestrus females, so that each male has to deposit enough sperm to compete with the presence of sperm from other males. For the chimpanzee, therefore, the volume of spermatogenic tissue and hence testis size must be far greater than in the gorilla or orangutan (Harcourt & Gardiner, 1994).

1.3.4.2. Epididymis

This structure consists of a long convoluted tube in all Hominoid species, divided into 3 parts: caput, corpus and cauda, where the maturation and adequate storage of the sperm cells occurs.

1.3.4.3. Vas deferens

In man the vas deferens is between 3 and 4 mm in diameter and about 38 cm long. In apes, comparable dimensions prevail, due to similar anatomical size and structure.

1.3.4.4. Seminal vesicles

These glands are generally elongated and lobulated and filled with small tubules supported by a connective tissue framework. They produce the bulk of the fluid portion of the ejaculate, fructose and prostaglandins (Short, 1979) and a fluid-coagulating substance. The seminal fluid is thought to have several functions: mechanical (transportation medium), vaginal pH buffer and survival of the spermatozoa. Chimpanzees have the largest seminal vesicles of all the Hominoid species, thus chimpanzee semen, upon ejaculation is almost entirely coagulated, and often will remain so. Gorilla semen is usually liquid, and the seminal vesicles

are smaller than those of man. The seminal vesicles of orangutans are larger than gorilla's and man's.

1.3.4.5. Prostate gland

This is a midline structure, surrounding completely the cephalic portion of the urethra. In man, the ducts of the seminal vesicles meet the vasa diferentia prior to penetrating the prostatic tissue, while in the chimpanzee the four ducts course separately through much of the prostate before eventually uniting to form the ejaculatory duct. The chimpanzee resembles man in the anatomy of the prostate; there are no well-defined cranial and caudal lobes.

1.3.4.6. Bulbo-urethral Glands

These glands occur in the orangutan, chimpanzee (Graham, 1970) and they have also been observed in the gorilla (Oelrich, 1978). The glands are not large, and the secretion has not been analysed. The lining epithelium is similar to that of the urethra.

1.3.4.7. Penis

Short (1979) provides a good review of the literature regarding penile appearance, dimensions and gross anatomy. All ape species possess a bony os penis, in contrast to man, who has no such element. The long body hair of the gorilla and orangutan, coupled with small penile size makes the organ essentially invisible unless searched for when the animal is anaesthetized. During erection, penile engorgement in the orangutan is visible, but that of the gorilla remains inconspicuous.

The chimpanzee penis is much larger and lighter in colour, hence it can be seen easily especially against the background of dark body hair. The chimpanzee is unique having no evidence of a glans penis, however there is erectile tissue in the penis of all the apes. In the chimpanzee, the corpus cavernosum begins essentially where the os penis ends (about 6 mm behind the tip) and continues dorsally along the entire length of the penis; below it is the corpus spongiosum.

2. Materials and Methods

The present study consisted in a database analysis of ultrasound examinations performed essentially by Prof. Dr. Thomas Hildebrandt, Dr. Frank Göritz and Dr. Robert Hermes since 1995; and one examination performed/assisted by the author of this dissertation, with these doctors' supervision in May 2012.

The IZW reproduction management group database was composed of thousands of recorded image sources from digital to VHS tapes including sonographic images from all the animals examined by them since the beginning of their careers.

2.1. Subjects

Regarding great apes, the IZW film archive database included ultrasound examinations from 18 gorillas, 6 orangutans, 2 bonobos and 3 chimpanzees from European, American and South-African Zoos. General information about the examined subjects can be seen on the Table 3 below.

The age categories adopted to characterize the subjects were: Adolescent (8-11 years) (Sugiyama, 1994), Adult (12-30 years) and Aged (>30 years) (Wharton & Thompson, 2000).

Data on anamnesis of the subjects and on their clinical and reproductive management throughout each case, was dependent on correspondence with the respective Zoo veterinarians, and thus may be incomplete or undisclosed.

According to this study supervisor, the clinical signs for most reproductive diseases in great apes are often unspecific (e.g. depression, weight loss); moreover, signs like pelvic pain and mild menorrhagia are hard to detect given their intrinsic undisclosed nature and dark skin/coat of these animals.

Table 3. General information about the examined subjects

Species	Name	Stud book no.	Sex (F/ M)	Age at exam (Years)	Detection of US lesions (Y/N)	Offspring prior to exam (Y/N)	Date of exam	Zoo Location
Gorilla *Gorilla g. gorilla*	Zaire	0557	F	32	Y	Y	July 06	London
	Liesel	0649	F	31	Y	Y	Sep 08	Budapest
	Haloko	0393	F	28	Y	Y	0ct 95	Washington
	Kishka	0681	F	28	Y	Y	July 07	Jersey
	Sakina	0922	F	21	Y	N	July 07	Jersey
	Amani	0899	F	26	Y	N	May 11	Givskud
	Jitu	0834	M	25	Y	N	Mar 08	Blackpool
	Zsazsa	0941	F	24	N	Y	Feb 10	Frankfurt
	Bongo jr. (Bobby)	1258	M	23	Y	N	July 06	London
	Yakwanza	0855	M	22	Y	Y	Aug 06	Jersey
	Dufte	0558	F	21	Y	Y	Feb 95	Berlin
	Dian	1091	F	21	Y	N	Feb 10	Frankfurt
	Mike	1097	M	20	N	N	Oct 09	La Palmyre
	Nyuki	1098	M	19	N	N	Oct 09	La Palmyre
	Kolo	0936	F	14	Y	N	May 00	Stuttgart
	Bahasha	1328	F	12	N	N	Aug 06	Jersey
	Mutasi	1316	F	8	Y	N	Oct 02	Stuttgart
	Asante	893	F	19	N	N	May 12	Twycross
Bonobo *Pan paniscus*	Zorba	168	M	26	N	Y	Feb 07	Stuttgart
	Ana Neema	?	F	10	N	Y	Nov 05	Milwaukee
Chimpanzee *Pan troglodytes*	Jenny	?	F	54	Y	Y	Dec 11	Pretoria
	Lillie	11888	F	26	Y	N	Dec 06	Berlin
	Phil	12883	M	11	N	N	Feb 08	Whipsnade
Bornean orangutan *Pongo pygmaeus*	Rawit†	1778	F	25	Y	N	Aug 05	Berlin
	Conny	2260	F	17	Y	N	Feb 07	Stuttgart
	Karolin	1349	F	26/27	Y	Y	May 00/ Dec 00	Stuttgart
	Moni	1609	F	22/23	Y	Y	May 00/ Dec 00	Stuttgart
Sumatran orangutan *Pongo abelii*	Tiba	1499	F	33	Y	Y	Oct 09	La Palmyre
	Sekara	2362	F	19	Y	Y	Aug 09	Perth

2.2. Fertility assessment terminology

Considering the terminology used to address the fertility prognosis of the examined subjects on this dissertation, it is important to mention that a wide debate exists on this topic, and so far researchers and clinicians are yet to reach a consensus (Gurunath, Pandian, Anderson & Bhattacharya, 2011).

In humans, the current clinical definition of infertility is considered to be 12 months of unwanted non-conception <with unprotected intercourse in the fertile phase of the menstrual cycle, whereas the term “subfertility” is not strictly established, covering disorders ranging from sterility to (nearly) normal fertility (Habbema, Collins, Leridon, Evers & Lunenfeld, 2004). In Zoo and wild life medicine, since the above definitions might not adequately apply, the reproductive terminology is also lacking uniformity. In this work, the terms “infertility” and “subfertility” were avoided, resorting to the term “impaired fertility” instead, simply meaning reduced ability to spontaneously produce offspring. However, towards the conclusions, the following system of ordered prognostic categories regarding the spontaneous probability of conception was used, based on Habbema et al. (2004): “fertility most likely uncompromised”, “fertility may be compromised” and“fertility most likely compromised”.

2.3. Equipment

Up until 2007, all the examinations with ultrasound scanning systems were performed in the portable Hitachi model (CS 9100 Oculus, Picker International GmbH, Espenkamp D-32339, Germany) and from then on, the Voluson i (Voluson i, GE Medical Systems, Zipf, Austria – Fig 7 A) was introduced, providing the database with 3D and 4D views into the reproductive tract. Only one examination was performed with a stationary E6 system (Voluson E6, GE Medical Systems, Zipf, Austria – Fig 7 B).

The systems were usually equipped with an abdominal 5-2 MHz curved array transducer and a vaginal 9-5 MHz intraoperative probe inserted into a customized probe extension. In males, ultrasonography of scrotum was achieved through a transcutaneous handheld 7.5 MHz curved transducer and the visualization of the accessory glands was performed transrectally using a vaginal transducer, due to lack of a proper prostate probe. (Note: prostate transducers have a downwards beam, instead of a “from the tip” beam, like vaginal probes).

Fig 7. Ultrasound scanning systems.

2.4. Scanning techniques

Overall, the following rules for ultrasonographic scanning were applied (Jaffe, Pierson & Abramowicz, 1994):

- The chosen transducer was always the one with the highest frequency that could adequately penetrate target structures.
- All structures were scanned in two planes (90 degrees to each other, i.e., longitudinal and transverse to the organ)
- The ultrasound beam was kept perpendicular to the target organ whenever possible for better resolution, except for Doppler mode. For D-mode imaging the angle of the sound beam transducer was less than 60 degrees.

Great apes were best scanned in dorsal recumbency for transabdominal ultrasound examination and in lateral recumbency for transvaginal and transrectal examinations.

Initially, the animal was placed on dorsal recumbency for endotracheal intubation. During this period, shaving of the pelvic area was performed for time saving purposes.

Ultrasound gel (Aquasonic 100, Parker Laboratories, Orange, New Jersey 07050, USA) was used in every examination as an acoustic coupling medium.

For all examinations, the probes were manually guided over the structures of interest.

Every rectal examination required a previous an enema with saline solution at 37ºC, for faeces removal. In humans, a protection condom is required when a probe is inserted vaginally or rectally, however, this measure was not taken in the IZW examinations in order to obtain a better quality image with improved resolution; thus, all instruments were thoroughly disinfected instead.

Nearly all ultrasonographic structures were measured during the examination resorting to the built-in calipers of the scanning system tools. Exceptionally, some unrecognized lesions had to be measured from sonographic pictures post-examination using the program cell^D by © Olympus Soft Imaging Solutions GmbH.

In some cases, endocrine analyses were performed on the blood serum, faeces and urine as complementary diagnostic aids for ultrasonographic findings and vice versa.

2.5. Anaesthesia Protocol

Ultrasound examinations in great apes usually require general anaesthesia, and the chosen protocol varies with individual preference and with the anaesthetic methods recommended at the time. The wide range of existing anaesthetic protocols is especially highlighted by the fact that in some Zoological Institutions, such as Disney's Animal Kingdom, it is common practice the training of the great apes for cooperation during the administration of the anaesthetic, reducing the stress associated with the anaesthesia event and total drug intake (Videan et al., 2005).

Thus, since this database analysis is spanned through 20 years and many of the anaesthesia protocols that were applied have been outdated or are very much disparate/uneven, the present work will describe in more detail, a single distance immobilization protocol, Table 4. This protocol in particular is from Dr. Sarah Chapman, head veterinarian of the Twycross Zoo, and it was used for the examination of the female gorilla Asante, in 2012, assisted by the author.

Table 4. Distance immobilization protocol utilized by Dr. Sarah Chapman.

Phase	Drug	Via	Time
Pre-medication	Midazolam 0.5 mg/kg	PO	09:15
Induction	1 mg/kg Zoletil® 0.02 mg/kg Medetomidine	IM by dart	09:35
Maintenance	Isoflourane 1.5-2.5% 100% oxygen at 1.5 L per minute	Endotracheal tube	09:35
Reversal	Atipamezole 0.1 mg/kg	IM	11:00

A substitution electrolyte solution (400 mL Hartmanns total, at a 10 ml/kg/h infusion rate) was provided during the procedure through a catheter in left cephalic vein. Monitoring was carried out by, Dr. Chapman and nurse Bridget Fry, who reported no abnormal occurrences: heart rate was kept in the 62-90 bpm range, oxygen saturation 86-98%, CO_2 27-34 mm Hg and respiratory frequency around 12-30 per minute.

Zoletil® (or Telazol) is a fixed-ratio combination of the benzodiazepine tranquilizer zolazepam, with the dissociative anaesthetic tiletamine, and has been widely used in primates with published doses for chimpanzees, gorillas and orangutans, Table 5.

Table 5. Range of dosages of injectable anaesthetic induction and reversal agents used in great apes (Sleeman, 2007).

Drug	Chimpanzee	Gorilla	Orangutan
Induction Agents			
Ketamine	5–20	6–10	6–10
Ketamine/xylazine	10–20/1		5–7/1–1.4
Ketamine/medetomidine	2–5/.02–.05	2–5/.02–.05	Use reported
Ketamine/midazolam		9/0.05	1–2/0.03
Telazol	2–6	2–6	2–6.9
Telazol/medetomidine	1.25/0.03–0.04		0.8–2.3/0.02–0.06
Reversal Agents			
Atipamezole	0.1–0.5 (intramuscular/ intravenous)	(all species)	
Yohimbine	0.125–0.25	(all species)	
Flumazenil	0.02–0.1 (intravenous)	(all species)	
Naloxone	0.02 (intramuscular/ intravenous)	(all species)	

Dosages are listed as mg/kg for intramuscular injection unless stated.

According to studies by Fahlman, Bosi & Nyman (2006), Zoletil® alone or in combination with α2 agonist (medetomidine) produces a smooth rapid induction and stable cardiopulmonary parameters (except for low blood pressure registered in chimpanzees).

Intubation is usually a requirement in great apes, especially in gorillas, due to risk of aspiration pneumonia from vomiting during the anaesthesia. Intubation can be very complicated, because the plane of anaesthesia achieved by many of the common utilized induction agents (Table 5) may not be adequate for intubation, sometimes requiring the use of a larynx mask (Sleeman, 2007).

For unidentified reasons, a previous fasting of the animal was not performed in the described Twycross case, but fortunately, the intubation was successful before vomiting occurred. With the endotracheal tube in place, upon signs of impending emesis, the animal was quickly turned to face the floor, in order for the gastrointestinal content to be easily expelled.

3. Results and Discussion

3.1. Sonomorphology of the genital tract of the female gorilla

Due to the close taxonomic relationship of human and great apes, techniques used for humans are commonly applied to these animals, but anatomical differences can sometimes pose problems. Knowledge of the appearance of healthy genital tract structures is necessary for clear recognition of pathologic alterations. Thus, the sonomorphology of the great apes reproductive tract is presented. The images are taken from the IZW reproduction management group databank, and the structure recognition was based on the clinical knowledge of this study supervisors', as well as on a few articles about the reproductive sonomorphology of women (Jaffe et al., 1994) and non-human primate females, including great apes (Ball et al., 2000; Calle et al., 2000; Tarantal, 2005).

While ultrasound imaging of the human female reproductive tract is typically conducted by a transabdominal or transvaginal approach, captive female great apes are best evaluated by transrectal ultrasonography (TRUS). In these animals, transabdominal ultrasonography, (TAUS) is frequently limited by the presence of abundant gastrointestinal gas/ingesta and abdominal fat; while transvaginal ultrasonography (TVUS) is restricted by a short muscular vagina with poor distensibility. Nevertheless, the most reliable assessment of the female gorilla reproductive tract is achieved by performing TRUS and TAUS together. Whereas TRUS provides a more detailed view of the reproductive organs, TAUS allows a better visualization of structures' size and global relations to other pelvic structures.

Note: all images in the female sonomorphology chapter are from female gorilla subjects.

3.1.1. Transvaginal approach and 3D TVUS Imaging

As mentioned previously, transvaginal sonography has limited use in great apes, thus, this imaging technique was seldom used by the IZW group. In the few cases that it was used, the reason was the presence of an unclear abnormal structure in a poorly identifiable location or with poorly delimited contour through TRUS; it was thought that resorting to TVUS and obtaining a different, more detailed view of the lesion could be beneficial for the diagnosis. Thus, few TVUS examinations were found on the IZW database and even less with normal findings (Fig 8 A). The main advantage of transvaginal ultrasonography is the increased image quality obtained from using a higher frequency transducer that is placed close to the cervix.

In the same regard, though 3D ultrasonography can be used in every approach (transrectal, transabdominal and transvaginal), in humans 3D TVUS (Fig 8 B) is considered the best view to evaluate endocavital structures, because the proximity coupled with the visualization of the 3 orthogonal planes simultaneously, allows a better delineation of the suspicious structure's

location and position in relation to the cavity delivering a better overview of the condition and avoiding diagnostic pitfalls (Momtaz, Ebrashy & Marzouk, 2007).

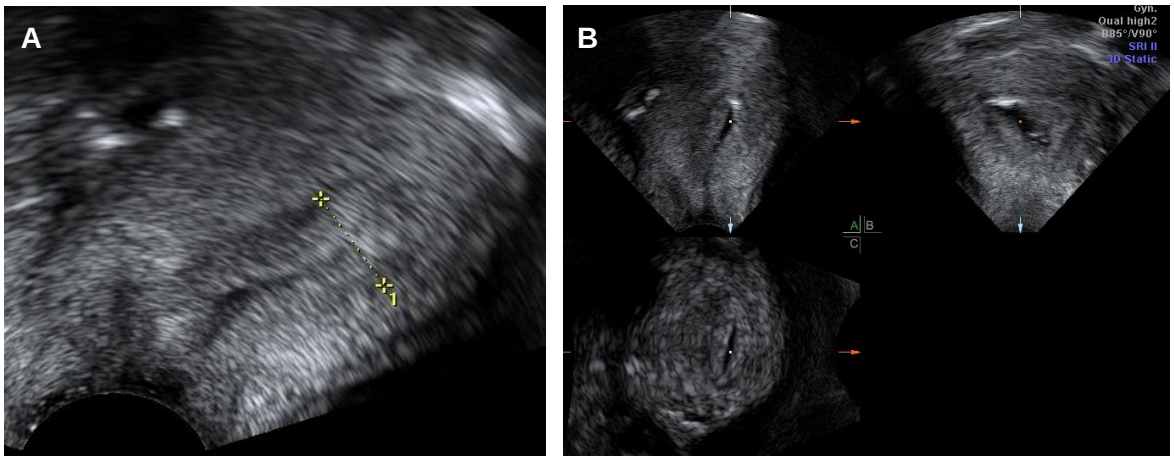

Fig 8. (A) uterus seen from a transvaginal probe - crosshairs mark the endometrium with a tiny amout of fluid; (B) three-dimensional ultrasound depicting multiplanar display of the uterus with internal debris typical during menses.

3.1.2. Transrectal approach

Transrectal ultrasonography, on the other hand, is the standard technique for evaluating the great ape reproductive tract and all of the animals were scanned this way. The examinations of the animals with a normal genital tract appearance were selected for the description of the sonomorphology.

The vaginal lumen was visualised longitudinally as a bright linear echo, surrounded by echogenic tubular walls posterior to the anechoic bladder (Fig 9 A).

The cervix consisted of a narrow passage between thick walls, in a bottleneck shape, followed by the endometrial stripe up until the uterine fundus (Fig 9 B).

The uterus was typically found on the midline, however, the uterine body is highly mobile and can be flexed either to the right, left, ventral (anteflexed) or dorsal (retroflexed) in relation to the cervix. The uterus may also be found in other locations due to abdominal or pelvic adhesions.

Ultrasonographically, the uterus appeared as a pear shaped organ with a rounded fundus. As a virtual cavity, the uterine lumen can be perceived by the opposing surfaces of its lining forming a stripe of varying thickness within the centre of the organ, the endometrium (Fig 9 C).

The presence of intra-uterine fluid in a non-gravid and non-menstruating female was usually an indication of pathology, such as haematometra, abortion, cervical obstruction, etc. Uterine flushing was performed occasionally to fill in the virtual cavity with saline solution and better observe the endometrial contour.

The uterus of great apes, as in humans, is supposed to have a relatively homogenous echo-texture, with echoes of medium intensity. The inner myometrial layer (junctional zone), which

has less connective tissue and more compact smooth muscle than the outer layers (outer myometrium and perimetrium), is the least echogenic of the three layers. The outer myometrium may contain small, ultrasonographically visible vessels that represent a venous plexus.

The overall uterine length was evaluated in the long axis from the fundus to the cervix (external os). The depth was measured from the anterior to the posterior wall and perpendicular to the length.

The TRUS exam allowed the visualization of the adnexa, which included the ovaries, fallopian tubes, ligaments, and mesosalpinges, but only the ovaries were routinely visualized. The fallopian tubes were found as minute adnexal structures not consistently visualized in non-pathological ultrasonography, but could be seen occasionally in physiological conditions in the presence of intra-abdominal fat (Fig 9 D) or intraperitoneal fluid released with ovulation. Uterine flushing also permitted the assessment of tubal patency by observing fluid collection outside the uterine cavity after saline injection.

The ovaries appeared as almond shaped structures, similar to that described in primates. They had a typically coarse low level echo pattern interrupted with anechoic areas that represented developing follicles or functional cysts (Fig 9 D, E, F).

Vascularisation of the ovaries can be seen on Fig 9 F, which displays the vessels supplying the follicles peripherally.

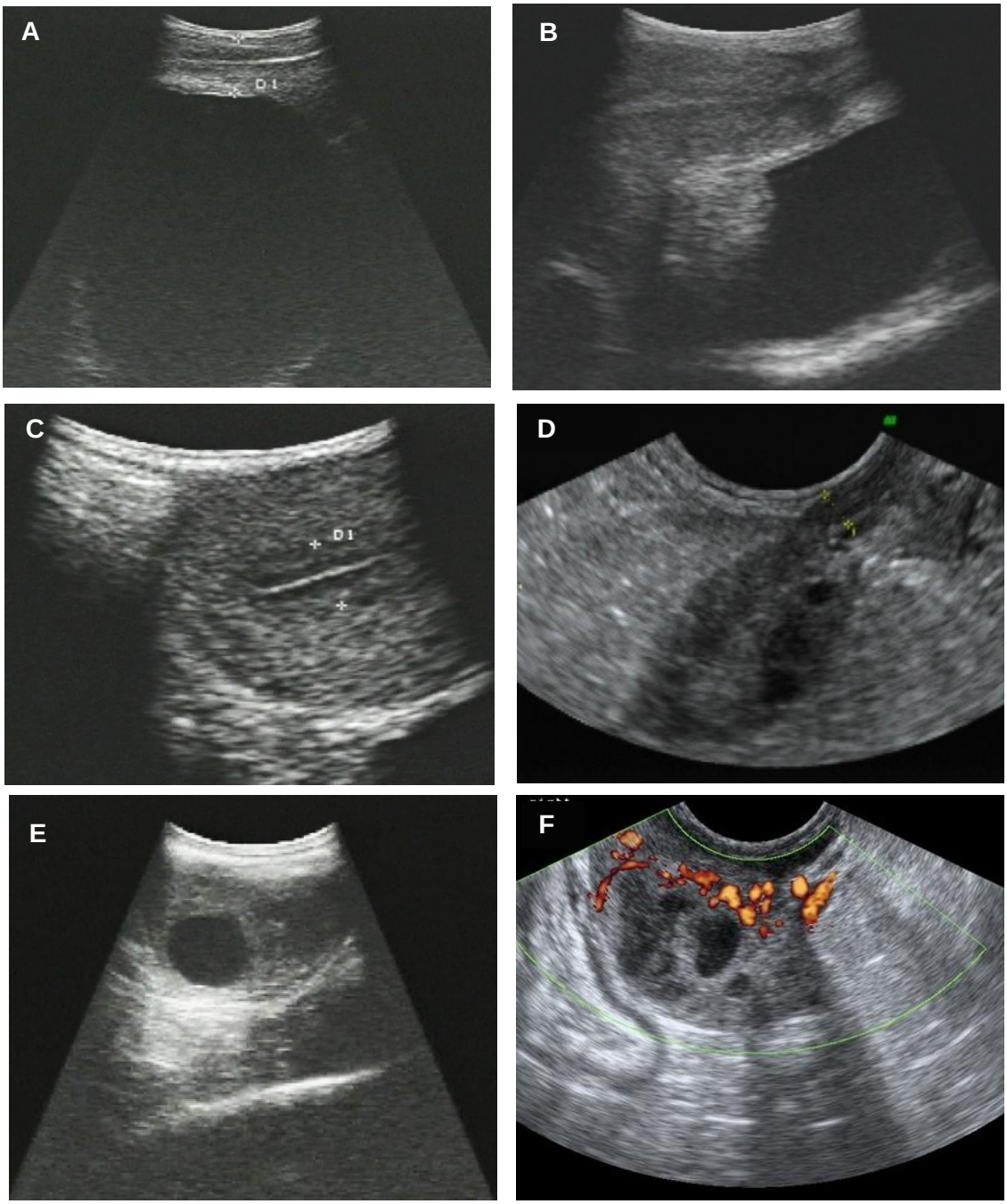

Fig 9. (A) Longitudinal section through the vagina, the white stripe corresponds to the vaginal interface; (B) cervix; (C) uterus, showing the acoustic properties of the uterine layers (endometrium and myometrium); (D) oviduct (usually not visible); (E) ovary, displaying a graafian follicle; (F) ovarian vascularization viewed through power Doppler.

3.1.3. Transabdominal approach

In TAUS, the bladder was situated over the uterus, and the degree of bladder fullness affected the uterine position. Ideally, scans should be made to subjects with a fluid-filled bladder, since it positions the uterus in a more horizontal plane and right in the middle focal range of the transducer.

The uterus on transverse TAUS is more or less an oval organ, where all the uterine layers can be seen and measured individually, as well as its total depth and width (Fig 10 A). Length can be measured by placing the probe longitudinally in the abdomen (Fig 10 B).

The cervix could be seen in cross section as the narrowest part of the uterus (Fig 10 C).

The ovaries are normally on either side of the uterus, slightly below the uterine fundus, but due to the flexibility of the supporting round ligament, they can assume other positions in the pelvis (Fig 10D).

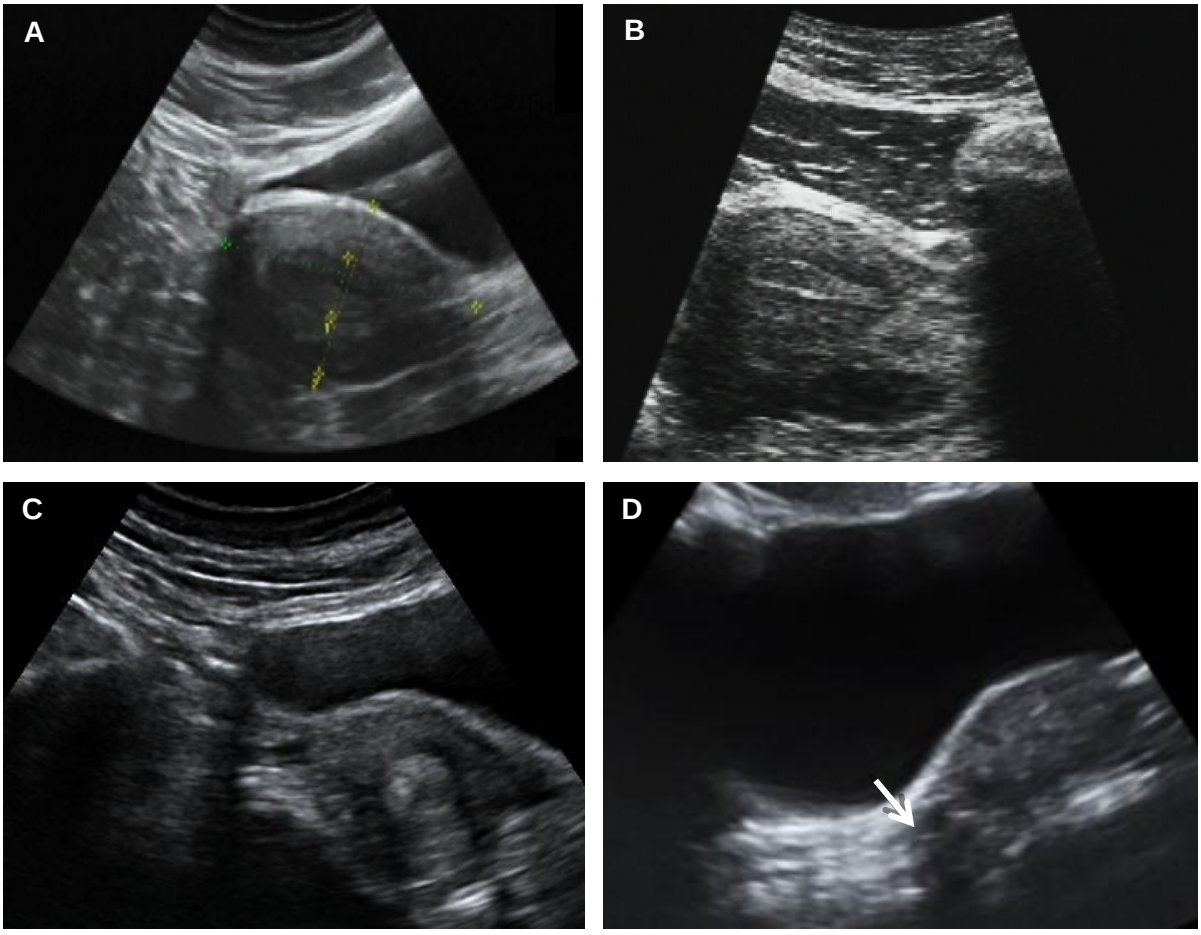

Fig 10. (A) Cross section of the uterus of a gorilla and its concentric layers (crosshairs); (B) slight longitudinal view of the uterus of a gorilla; (C) cervix of a gorilla in cross section; (D) ovary (arrow) beneath the bladder and lateral to the uterus.

3.1.4. The endometrium during the menstrual cycle

The endometrium was consistently evaluated in both planes (longitudinal and transverse). Its thickness varied according to the phase of the menstrual cycle. An increase of thickness can be seen throughout the follicular (proliferative) phase in response to the rising of serum estrogen concentration. Thus, in the first days of the proliferative phase, the endometrial stripe is linear, thin and not very echogenic in relation to normal myometrium (Fig 11 A).

In the days preceding ovulation, a trilaminar pattern is described in humans and in non-human primates (Ortiz et al., 2005) and in gorillas (Calle et al., 2000). In this study, a nearly perfect trilaminar pattern was recognized, during the late proliferative phase endometrium (Fig 11 B). During the luteal (secretory) phase, high serum progesterone levels causes the endometrium to transform into a secretory histological pattern, characterized by increased echogenicity, thickness and loss of the trilaminar pattern (Fig 11 C).An abrupt decrease in endometrial thickness corresponds to the occurrence of menses: small quantities of hypoechoic fluid and debris can be seen in the uterine cavity and myometrial contractions are frequent.

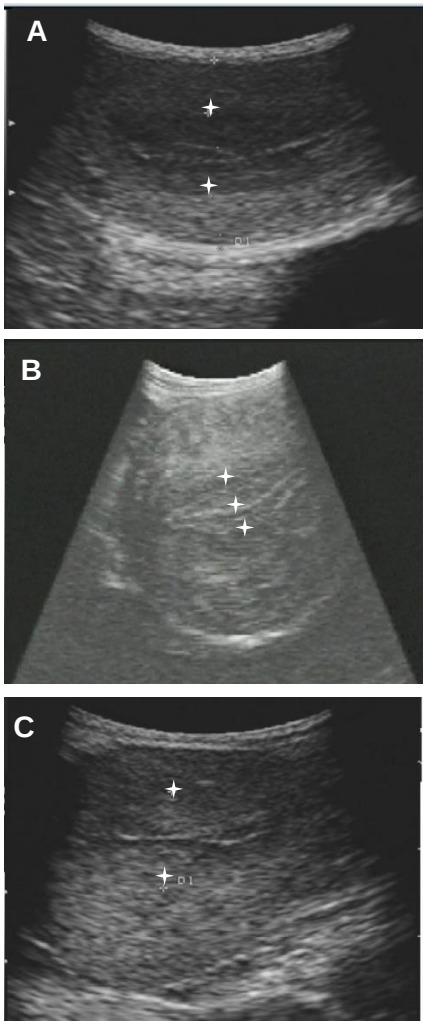

Fig 11. Endometrial US pattern reflective of the hormonally-induced progression of the menstrual cycle: (A) proliferative endometrium, hypoechogenic relatively to the myometrium (crosshairs); (B) trilaminar pattern (crosshairs) corresponding to late proliferative endometrium; (C) echogenic secretory endometrium (crosshairs).

3.1.5. Normal anatomic parameters

Table 6 displays the average size and the standard deviation, in brackets, of the ovaries and uterus of animals without relevant anatomic alterations, and also the endometrial thickness amplitude.

Both ovaries were considered in animals with normal adnexa (including functional cysts). However, in the presence of severe ovarian pathology, the affected ovary was rejected for mean calculation purposes and the contralateral ovary was only considered if its ultrasonographic appearance was normal. Endometrial thickness included both endometrial layers together, during all menstrual cycle phases. Animals with intramural leiomyomas not distorting the uterine cavity were considered for the measurement of endometrial thickness and uterine size. Though endometrial thickness was considered from an animal with suspicion of diffuse adenomyosis, uterus size was not.

Eventually, the sample size (N) was larger than the number of animals examined due to the occurrence of double examinations (2x).

Table 6. Normal US anatomic parameters of the female reproductive organs.

Structure	Genus	Measurements (cm)	N	Observations
Ovary	*Gorilla*	1.57 (±0.54) x 2.46 (±0.82)	17	Width x length from 10 animals (Zaire, Liesel, Kishka, Kolo, Haloko Bahasha, Dufte, Mutasi, Dian and Asante)
	Pongo	1.44 (±0.58) x 2.38 (±0.99)	16	Width x length from 6 animals (Karolin 2x, Moni 2x, Conny, Rawit, Tiba, Sekara)
	Pan	1.20 (±0.22) x 2.24 (±0.62)	3	Width x length from Lillie's ovaries and only one very inactive ovary from Jenny
Uterus	*Gorilla*	4 (±0.99) x 5.81 (±1.51)	8	Width x length from 8 animals (Kolo, Zaire, Kishka, Mutasi, Dufte, Haloko, Asante and Amani)
	Pongo	2.90 (±0.70) x 5.01 (±0.83)	8	Width and length from 6 animals (Rawit, Karolin 2x, Moni 2x, Conny, Sekara and Tiba)
	Pan	3.61 x 5.2	1	Width and length from Lillie
Endometrium	*Gorilla*	Max: 2.07 Min:0.82	11	Thickness from 10 animals (Kolo, Sakina, Bahasha, Kishka, Liesel 2x, Zaire, Dufte, Haloko, Amani and Asante)
	Pongo	Max: 1.41 Min: 0.5	8	Thickness from 6 animals (Rawit, Karolin 2x, Moni 2x, Conny, Sekara and Tiba)
	Pan	1.03	1	Thickness from Lillie

By analysing Table 6 it can be perceived that the *Gorilla* genus was the one with the largest uterine and ovarian size, and also with wider endometrial thickness variation in comparison to *Pongo* and *Pan*.

The uterus size of *Pan* was larger than *Pongo*'s, but the ovaries of *Pongo* were larger than *Pan*'s by a small difference. This data was compatible with the existing literature, where the ovaries of chimpanzees are reported to be smaller than those of humans and gorillas' similar to humans' (Wislocki, 1932; Schwartz, 2005).

Endometrial thickness in women varies from 0.2 to 1.6 cm, and when the upper limit is exceeded, suspicion of endometrial hyperplasia or endometrial carcinoma follows (Nalaboff, Pellerit & Ben-Levi, 2001). As far as we know, no such limits were defined for great apes. Since gorillas have larger uterus than humans, a thickness of 2.07 cm was not considered pathological.

3.2. Sonomorphology of the male great ape genital tract

Literature on the sonomorphology of the reproductive tract of the male great ape is scarce, with minor descriptions found only in the work of Dahl, Gould & Nadler (1993).

However, there were several human and non-human primate ultrasound texts that provided good reference material for this study, particularly Dogra, Gottlieb, Oka & Rubens(2003) scrotal ultrasound guidelines for human medicine and Amory et al. (2012) sonographic testicular anatomy of the St. Kitts vervet monkey.

3.2.1. Transrectal approach

The prostate in great apes was found to have an irregular ellipsoid shape similar to the ultrasonic appearance of a mature human prostate.

The prostatic parenchyma showed a fine and uniform hypoechogenic appearance well delineated from the other pelvic structures (Fig 12 A).

In both transverse and sagittal planes, the seminal vesicles, with low echogenicity, were identified on the dorsal surface of the urinary bladder connecting to the base of the prostate (Fig 12 B).

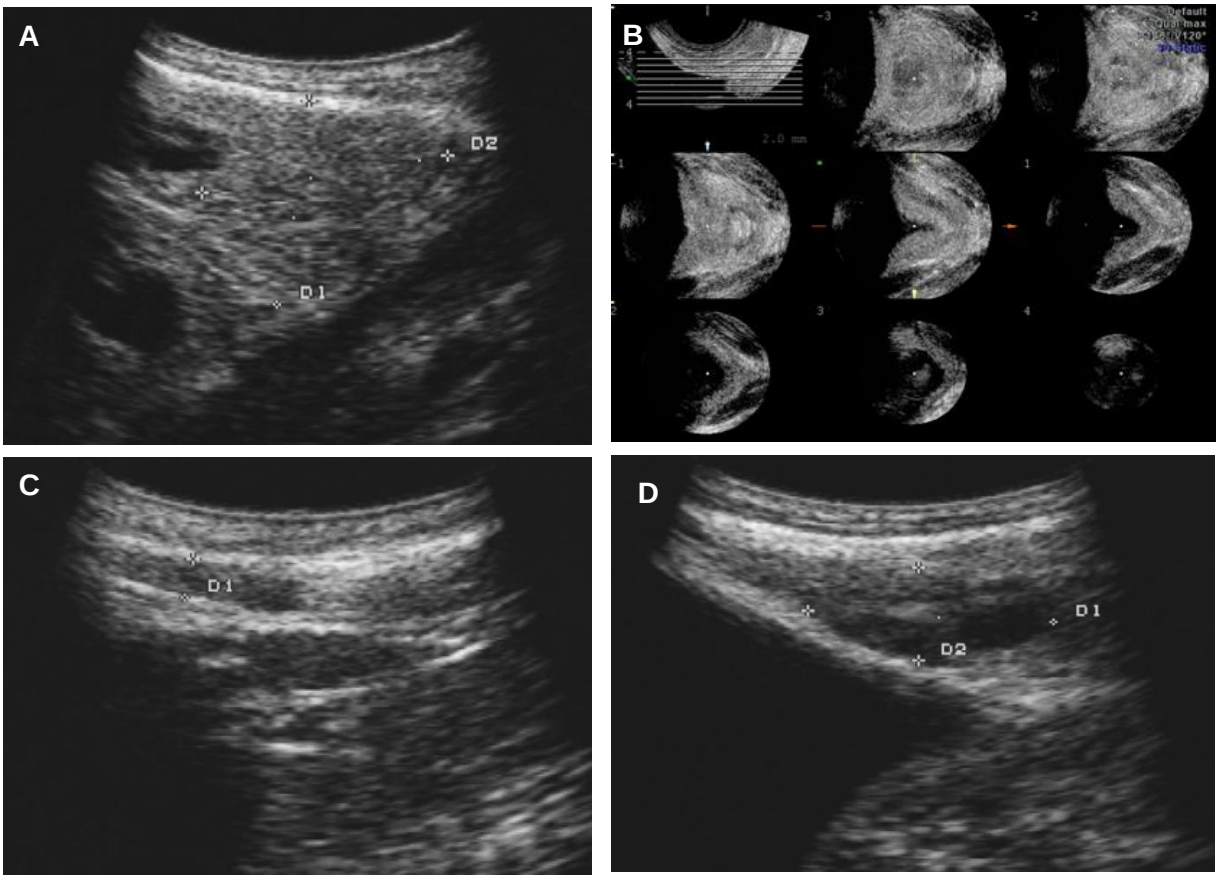

Fig 12. (A) Prostate lobe of a gorilla (crosshairs); (B) prostate in tomographic ultrasound imaging (TUI); (C) vas deferens of a gorilla, ampulla portion (crosshairs); (D) seminal vesicle of a gorilla (crosshairs).

3.2.2. Transcutaneous approach

The normal testes were found to have a homogeneous echo texture of medium-level intensity (Fig 13 A, G), and any inhomogeneous findings on ultrasound were considered abnormal. Each testis was surrounded by a fibrous band, the tunica albuginea, which was often not visualized in the absence of intrascrotal fluid.

The scrotal structures demonstrated by ultrasound were: the mediastinum testis and the epididymis (caput, corpus and cauda).

The mediastinum testis appeared as an echogenic linear structure on the midline (Fig 13 E and F); testicular perfusion was shown through Doppler colour flow ultrasonography consisting of a blood flow pattern from the capsular artery into the centripetal and centrifugal intratesticular rami, meeting on the mediastinum (Fig 13 B).

The epididymis was iso to hyperechoic comparing to the normal testis and had equal or diminished vascularity. The caput epididymis lied superior and lateral to the upper pole of the testicle and had the shape of a triangle (Fig 13 D). The normal epididymal body was narrow and it connected to the distal border of the testis by the cauda epididymis, which had a coarser echotexture than the testicle (Fig 13 C and F).

The penis was scanned occasionally and its appearance is shown in Fig 13 H.

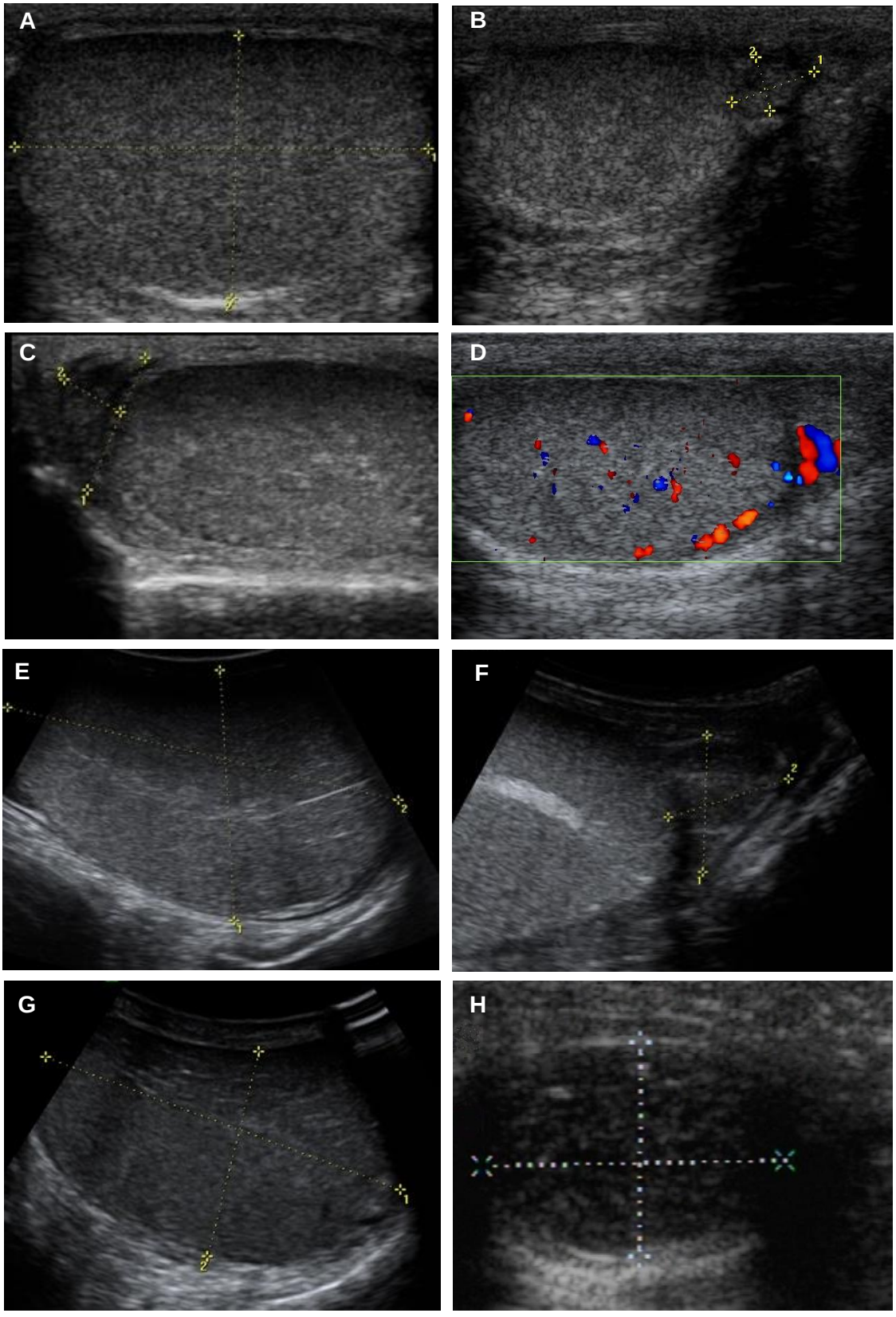

Fig 13. (A) Longitudinal view of testes of a gorilla (length and width in cm - 3.59 x 2.26); (B) normal vascularization of a gorila testis visualized through Doppler colour flow; (C) longitudinal section of cauda epidydimis of a gorilla (measurements in cm - 1.20 x 0.56); longitudinal section of the caput epidydimis of a gorilla; (E) longitudinal section of the testes of a chimpanzee (length and width in cm - 7.38 x 4.97); (F) longitudinal section of the cauda epidydimis of a chimpanzee (measurements in cm - 2.02 x 1.77); (G) longitudinal section of testes of a Bonobo (length and width in cm - 6.76 x 3.81); (H) transverse section of the penis of a gorilla.

3.2.3. Normal anatomic parameters

Table 7 presents the average size and standard deviation (in brackets) of the testes of gorillas, chimpanzees and bonobos scanned by the IZW group.

The sample from gorillas excluded testicles with intra-testicular lesions but accepted the contralateral testis for average calculation if its appearance was normal. The considered exemplars from Pan were both normal healthy testes.

Table 7. Normal US anatomic parameters of male reproductive organs.

Structure	Genus	Measurements (cm)	N	Observations
Testis	*Gorilla*	1.90 (±0.32) x 3.57 (±0.30)	7	Width and length of both testicles from Nyuki & Mike and only the contralateral testes of Kwanza, Jitu and Bobby
	Pan	4.42 (±0.58) x 6.98 (±0.35)	3	Width and length from both testes of Zorba and one of Phil's testis
Epididymis *Gorilla*	Cauda	0.67 (±0.25) x 1.02 (±0.17)	7	Width and length from both epididymis of Bobby, Jitu, Nyuki, and only the contralateral from Kwanza
	Caput	0.46 (±0.1) x 0.81 (±0.19)	7	
Epididymis *Pan*	Cauda	1.67 (±0.15) x 2.01 (±0.01)	2	Width and length from the epididymis of Zorba and Phil (only one each and Phil no caput)
	Caput	0.95 x 1.08	1	

The results in Table 7 show that the examined subjects from the *Gorilla* genus had a testes area, i.e. width x length, approximately 4,5 times smaller than from *Pan* and epididymides area circa 3 times smaller.

In the literature, the mean testicle size of the chimpanzee exceeds the gorilla's by a 3-fold (Collins, 1981).

Short (1979) reported a larger testicular size discrepancy between these genuses present in males of proven fertility: gorilla Samson (testes sizes 1.8 x 3.8 and 2.2 x3.5 cm) and chimpanzee Buttons (testes sizes 5.5 x 8.0 and 6.5 x 8.0) from Bristol Zoo, had a 6 fold difference in their testes measurements.

In both genuses the cauda epididymis was larger than the caput, contrary to what occurs in *Homo* (Dogra et al., 2003).

3.3. Uterine Sonopatophology

In this study 5 different types of uterine lesions were found, namely fibroid-like masses, cystic formations in the myometrium, uterine heterogenous echotexture, cystic formations in the cervix and solid cervical mass. The respective US images are presented together with the anamnesis contextualization in order to better assess the results. Systematic information on date, location and age of the subject at exam are indicated on Table 3 (page 18).

3.3.1. Fibroid-like masses

The animals found with this type of lesion were Kishka (28 years old) and Mutasi (8 years old), both female gorillas of reproductive age. Kishka, multipara, presented signs of depression in July 2007 and Mutasi, nulipara, was scanned for pregnancy check in October 2002.

Kishka's ultrasound examination showed a mixed mass, consisting in a solid hypoechogenic circumscribed area with small cysts within, located in the myometrium but reaching the subserous tissue (Fig 14 A). Mutasi had a simple solid hypoechogenic mass in the myometrium, causing little deformation of the organ (Fig 14 B).

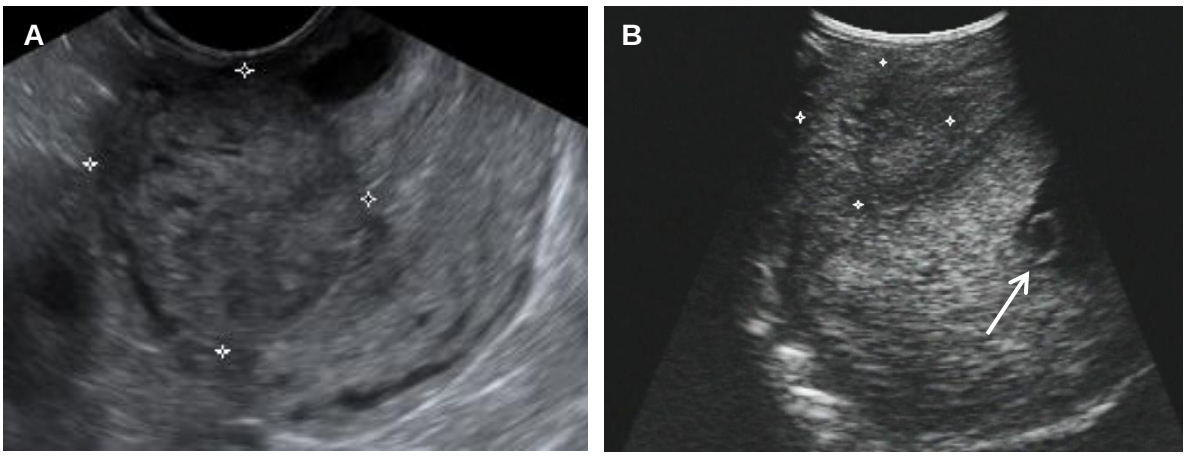

Fig 14. (A) Kisha's solid intramural/subserous mass (crosshairs - size in cm 4.78 x 5,69) with cysts; (B) Mutasi's intramural solid mass (crosshairs - size in cm 2.13 x 2,16) during pregnancy, yolk sac (arrow) indicating proximity to: 5 weeks pregnancy (humans – Asghar & Fatima, 2012)) and 18-20 days pregnancy in macaques (Tarantal, 2005).

Based upon the particular sonographic appearance and the clinical experience of the author's supervisor, it is suggested that the masses encountered in Mutasi and Kishka were uterine fibroids/Leiomyomas, which are benign fibroid tumours that arise from the smooth-muscle cells of the uterus (Videan, Satterfield, Buchl & Lammey, 2011).

Although other diagnoses (uterine malignant tumours, metastatic disease, adenomyoma) could not be excluded, the primary suggested diagnosis is supported by the fact that leiomyomata are described as ''common'' among nonhuman primates (Videan et al., 2011), existing reported cases in gorillas (Mylniczenko, Murrey, Smith, Sewall & Facchini, 2008; Stringer et al., 2010) and many more in chimpanzees (Brown et al., 2009; Silva et al., 2006).

According to Videan et al. (2011) leiomyomata in chimpanzees have a similar prevalence to that in humans, being 28.2% for all ages and increasing up to nearly 40% when only animals 30 years of age and older were considered.

This similarity suggests that leiomyoma development may follow analogous patterns in both humans and chimpanzees. Thus, Videan et al. (2011) anticipated that the risk factors for

women - increasing age, obesity, nulliparity, greater than 10 years since last birth, younger age at menarche and younger age of first birth - could also be associated with a higher risk of leiomyoma development in chimpanzees. Further studies are required to confirm these associations in great apes, in particular the relation between leiomyomata and obesity, since weight gain is an acknowledged problem across captive great apes populations (Videan et al., 2011).

Kishka, being 28 years old at the time of examination, nearly fits the age group most affected by this pathology (>30), and she also had a gap greater than 10 years since last birth (her last parturition had been in 1992, when her baby died of anoxia due to umbilical prolapse prior to delivery).

On the other hand, Mutasi's youth raises suspicion for a misdiagnosis, ever since Videan et al. (2011) leiomyomata studies excluded animals younger than 15 years of age for prevalence calculation. However, since she had a successful pregnancy at 8 years old, it can be speculated that she reached menarche at a very young age, which could increase her odds of developing uterine fibroid during adolescence.

Videan et al. (2011) reports that among women and chimpanzees, uterine leiomyomata are largely asymptomatic, yet, when symptomatic, the most common complaint is abnormally heavy or extended menstrual bleeding (i.e. menorrhagia) and more rarely: severe pelvic pain and uterine prolapse. Kishka and Mutasi did not present severe symptoms and it is not established if Kishka's previous umbilical prolapse could be related to her uterine lesion.

Leiomyomas can be often misdiagnosed through ultrasound examination, due to the occurrence of degenerative phenomena (cystic, hyaline, red) and peduncule formation, posing a diagnostic dilemma for the clinic (Baltarowich et al., 1988). Definitive diagnosis is frequently obtained only after hysterectomy, laparoscopy or biopsy.

Common ultrasonographic misdiagnoses of leiomyomata are: 1) adenomyosis (Levy et al., 2013); 2) endometrial hyperplasia & endometrial carcinoma (Madhok, Agarawal & Goel, 2012); 3) ovarian tumours (Low & Chong, 2004; Fried, Kenney, Stigers, Kacki & Buckley, 1996); 4) ectopic pregnancy & endometriosis (Baltarowich et al., 1988; Fried et al., 1996); 5) uterine malignant tumours (Exacoustos et al., 2007); 6) piometra and hidatidiform mole (Baltarowich et al., 1988).

3.3.2. Cystic formations in the myometrium

Two multiparous gorillas of relatively advanced reproductive age (Liesel, 31 and Kishka, 28) were found with cystic myometrial structrures:

Beyond Kishka's fibroid-like mass already discussed, Fig 14 A, she also showed an unaddressed large cyst in the periphery. This unaddressed lesion was more clearly visible transabdominally, Fig 15 A, consisting on a cystic structure of almost 1 cm in the outer myometrium, adjacent to a poorly circumscribed hypoechoic area.

Liesel was examined in September 2008 due to absence of sexual behaviour and weight loss. Her uterine scan showed only a cystic structure in the outer myometrium without a significantly altered surrounding area (Fig 15 B).

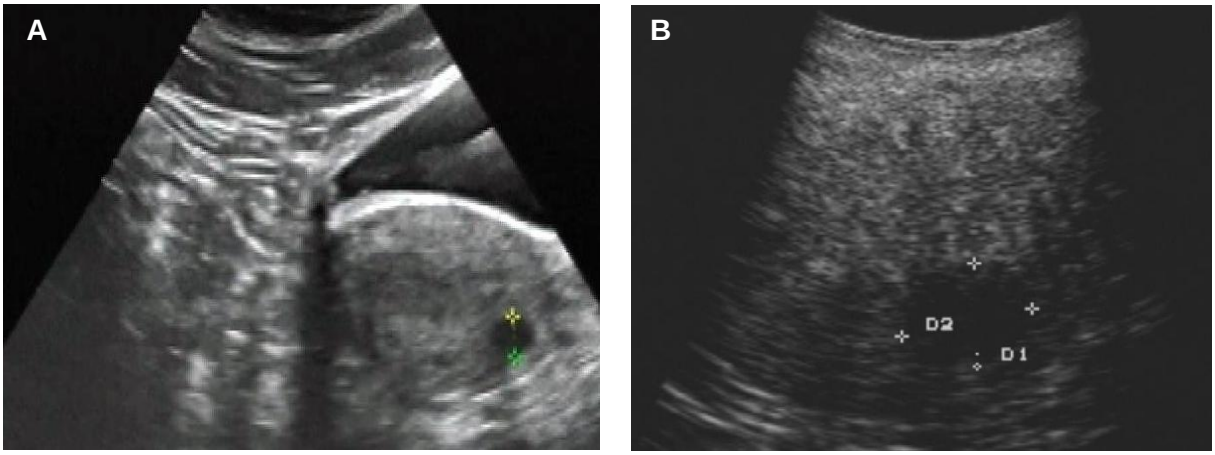

Fig 15. (A) Transabdominal view of uterus with a cystic structure (crosshairs - size in cm 0.99) in a slight heterogenous area in the myometrium; (B) single cystic structure (crosshairs - size in cm 1.12 x 1.53) in outer myometrium displayed in transrectal view.

In both cases, location of the cysts was confirmed to be the myometrium and the vascularisation of the lesion (not shown) was consistent with the normal uterus (not hypervascularised), which led to the suggestion of two primary diagnoses: cystic degenerated leiomyoma and adenomyosis, in detriment of endometrial hyperplasia, metritis, uterine malignant tumours and interstitial ectopic pregnancy.

As already mentioned, leiomyoma and adenomyosis ultrasonographic images are difficult to distinguish. The cysts resulting from the degeneration of a leiomyoma resemble adenomyotic myometrial cysts, since both are round anechoic spaces in the uterine wall. However, histologically, in adenomyosis the cysts correspond to fluid-filled dilated endometrial glands rather than degenerated muscle cells (Levy et al., 2013).

Videan et al. (2011) proposed a system for differentiating these diseases in chimpanzees, through the recognition of adenomyomata typical US characteristics, like poorly defined margins and an irregular mottled texture. In the US images of Fig 15, however, differentiation was difficult.

According to Taran, Weaver, Coddington & Stewart (2010) both diseases frequently coexist in humans, with approximately 15 to 57% of hysterectomized women uteri having adenomyosis and leiomyomas simultaneously. Having said that, since Kishka presented a spherical well-defined solid heterogenic lesion and a separated large myometrial cyst surrounded by an ill-defined hypoechoic area, she was suggested to have concomitant leiomyoma with adenomyosis.

As for Liesel's cyst, it was unclear whether it was a fully degenerated leiomyoma turned into a cyst (by lack of blood supply) or focal adenomyosis. Histopathology would be required to obtain a precise diagnosis.

Adenomyosis has been described in Baboons (Barrier, Malinowski, Dick, Hubbard & Bates, 2004) and also in the chimpanzee (Barrier et al., 2007) and orangutans (Graham, Hulst, Vogelnest, Fraser & Shilton, 2009; Munson & Montali, 1990).

As far as we know, there are no prevalence studies of this disease in great apes, and only one study researching the prevalence in rhesus macaques was found, describing a prevalence interval from 12 to 24% depending on whether the population was free-ranging or captive (DiGiacomo, 1977).

Studies with Baboons have demonstrated that adenomyosis is linked to impaired fertility, and with endometriosis (Barrier et al., 2004). However, in the reported cases of adenomyosis in chimpanzees, considerations regarding fertility were thought speculative given the few identified cases (Barrier et al., 2007).

As a final point, Kishka, proposed to have concomitant adenomyosis and a leiomyoma, was thought to be pregnant earlier in May 2012, given her positive pregnancy test results carried out at the Durrell Wildlife Conservation Trust in Jersey.

Only later, it was confirmed that she was not pregnant, though she was also mated by the fertile male, Badongo, who successfully impregnated her fellow mate, 24-year-old Hlala Kahilli. This evidence points to the likelihood of Kishka having her fertility impaired, due to, at least one, or a combination, of the pathologies she is suggested to have.

3.3.3. Uterine heterogenous echotexture

During this study it was possible to recognise a uterine heterogenous echo-texture and other alterations in the uterus of one female gorilla, Sakina, a nuliparous female suffering from social distress and failed attempts to become pregnant.

Sakina, daughter of Kishka, was examined by the IZW reproduction management group in July 2007, at the age of 21. Her US examination showed a relatively engorged uterus (taking into consideration that it is a nulliparous organ, measuring 9.44 x 5.95 x 7.43 cm) with spongy/heterogenous appearance. The myometrium of the cranial part of the uterus seemed slightly enlarged and there were submucosal myometrial cysts in the fundus region. Finally, the endometrium was not well defined (Fig 16 A).

She was also found to have small cystic formations in her ovaries, suggested to be cysts, due to her low LH and oestradiol levels (described further in 3.4.1.).

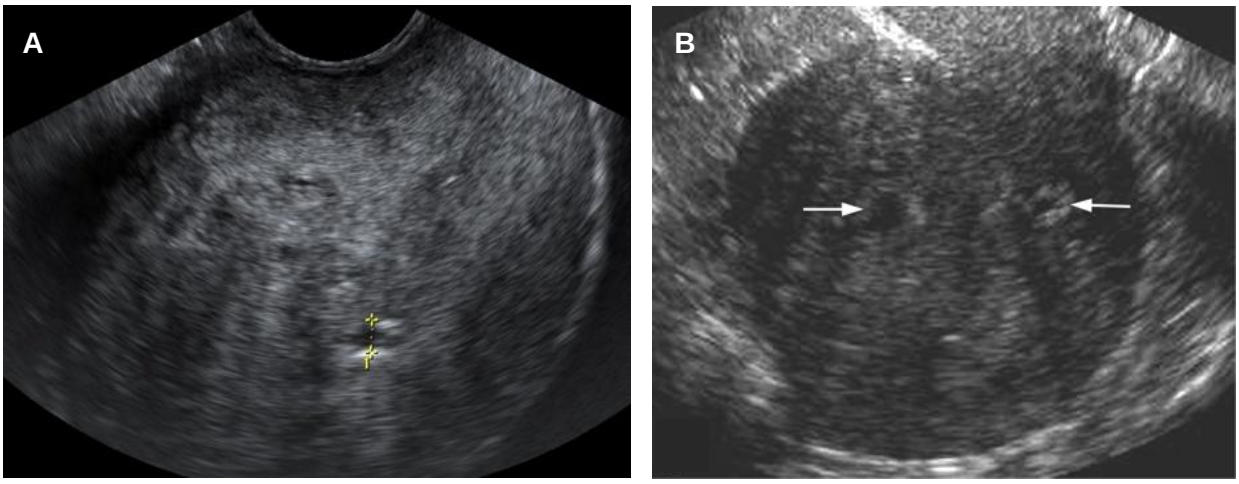

Fig 16. (A) Spongy/heterogenous appearance of uterus with small submucous myometrial cyst (crosshairs - size 0.39 cm) on a 21 year old gorilla; (B) myometrial cysts in heterogenous uterus of a woman, suggestive of adenomyosis (source of the image: Chopra et al., 2006).

Regarding her uterus, due to the abundance of factors (the following 5 underlined topics out of 7) that match the criteria established for adenomyosis detection in women – 1) uterine enlargement, 2) myometrial cysts, 3) assymetrical myometrial thickening, 4) heterogeneous echotexture , 5) indistinct endometrial/myometrial junction, 6) subendometrial echogenic linear striations, 7) thickening of the transition zone (Sakhel & Abuhamad, 2012) - uterine adenomyosis was suggested as primary diagnosis in Sakina's case, followed by the hardly disintinguishable cystic degenerated leiomyomata, uterine malignant tumours etc.

However, not fitting into the primary diagnosis of adenomyosis was the fact that Sakina was a young nulliparous female, and leiomyomas, unlike adenomyosis, are traditionally linked to nulliparity (Levy et al., 2013). Nevertheless, adenomyosis has been increasingly detected in fertility clinics: in women of late reproductive age, due to postponement of childbearing, or in younger women, often associated with endometriosis (Leyendecker, Kunz, Kissler & Wildt, 2006).

Sakina was euthanized on the 30th of August 2012, at the Gaia Zoo, in the Netherlands. She was found with severe pelvic pain and anorexia due to septic peritonitis caused by rupture of a pelvic abscess, which surgery and antibiotherapy could not repair.Her histopathology report described lesions typical of chronic adhesive peritonitis, large hemo-purulent fluid filled cysts at the site of the ovary, multifocal myometrial hemo-purulent cysts and interstitial proliferation of mesenchymal cells in the uterus; and the bacteriological culture of the fluid pointed to beta hemolytic *E. coli.*

Although her histopathology report made no mention of endometrial tissue or neoplastic cells in the myometrium, the presence of mesenchymal cells interstitially in the uterus could be taken as a minor clue to suggest adenomyosis, as this proliferation is consistent with the epithelial-mesenchymal transition theory for the development of adenomyosis (Chen et al.,

2010), but it could also be a sign of fibrosis or mesenchymal tumour (e.g endometrial stromal tumour - Toft & MacKenzie, 1975; or leiomyoma - Cooper & Gabrielson, 2007). A more detailed histopathology would be required for differentiation. Correspondence with the responsible histopathologist is underway.Since she had been previously suggested with polycystic ovaries and adenomyosis in 2007, it could be investigated if her pelvic abscess corresponded to infected ovaries (tubo-ovarian abscess) or a pyoadenomyoma.

Gorillas are described to have a high prevalence of intra-abdominal abscesses when compared to other reported diseases in this species (Mylniczenko, 2003). This author proposed the following possible causes for intra-abdominal abcessation in gorillas: 1) organ perforation due to enteritis, foreign body, trauma or reproductive tract infection, 2) iatrogenic causes and 3) nutritional deficiencies. Affected subjects are usually older, obese, females, with impaired fertility. See 3.4.4.pelvic masses discussion.

3.3.4. Cystic formations in the cervix

Some of the subjects examined by the IZW showed structures compatible with cysts in the cervical wall. Liesel, Dufte and Kishka were found to have several anechoic round structures below the isthmus of the uterus (Fig 17). In most cases, this finding was assessed to be secondary and not the main pathology.

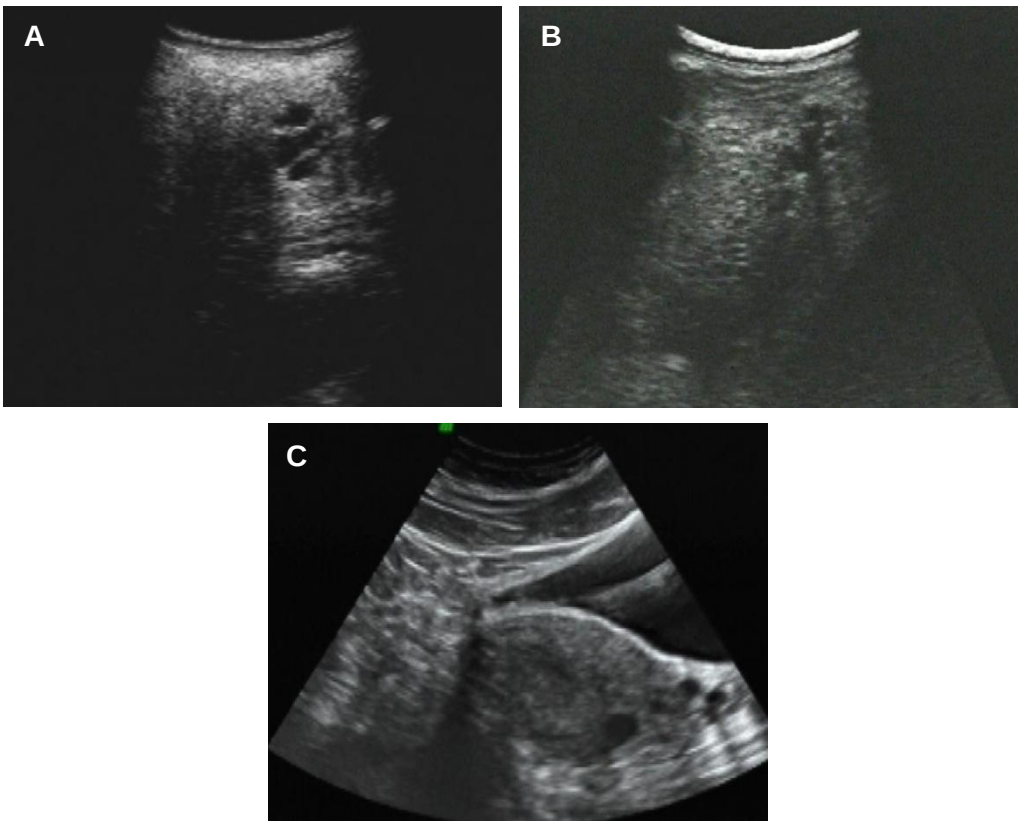

Fig 17 (A) & (B) multiple cysts in the cervical area, transrectal view (all measuring less than 1 cm); (C) longitudinal transabdominal ultrasound imaging showing multiple cysts (2 of which could be measured - diameters: 0.37 and 0.5 cm) situated anteriorly in the cervix.

After analysing some of the differential diagnoses: 1) cervical malignancy (rarely cystic, deeper cervical lesions with increased flow on Colour Doppler - Clement & Young, 1989); 2) Gartner cysts (usually vaginal -Dujardin, Schiettecatte, Verdries & de Mey, 2010); 3) endometrial polyps and hyperplasia (multicystic masses, rather than individualized wall cysts– Dujardin et al., 2010) and preliminarily excluding them, the lesions on Fig 17 were suggested to be nabothian cysts, while leiomyoma and adenomyosis remained as secondary diagnoses.

Nabothian cysts (retention cysts) are common gynaecological findings in humans (Sosnovski, Barenboim, Cohen & Bornstein, 2009). They have also been described as common in non-human-primates (Cooper & Gabrielson, 2007; Wood, 2008) and have been reported in gorillas (Calle et al., 2000) but there is lacking information about its prevalence in these animals. Retention cysts are usually a sequelae of the healing process of chronic cervicitis, postpartum cervix with ectropion (columnar epithelium migrating through cervical os) or of chemical irritants altering the vaginal acidity and flora. Patients on progestogenic therapy may develop nabothian cysts due to failure of the cyclic flow of cervical mucus (Slasky, 1982)

All of the above subjects with cervical cysts are parous which created the hypothesis of unrecognized post-partum ectropion or cervicitis leading to cystic formation.

3.3.5. Solid cervical mass

A hyperechogenic cervical mass was found in an aged chimpanzee, Jenny, along with trapped intra-uterine fluid (Fig 18 A, B). In December 2011, Jenny was examined in Pretoria, due to vague symptoms and a little blood loss from the vulva, initially thought to be a wound. Catheterization was attempted to extract the fluid from her uterine cavity but it was not successful. Her lesion seemed to extend to the uterine corpus.

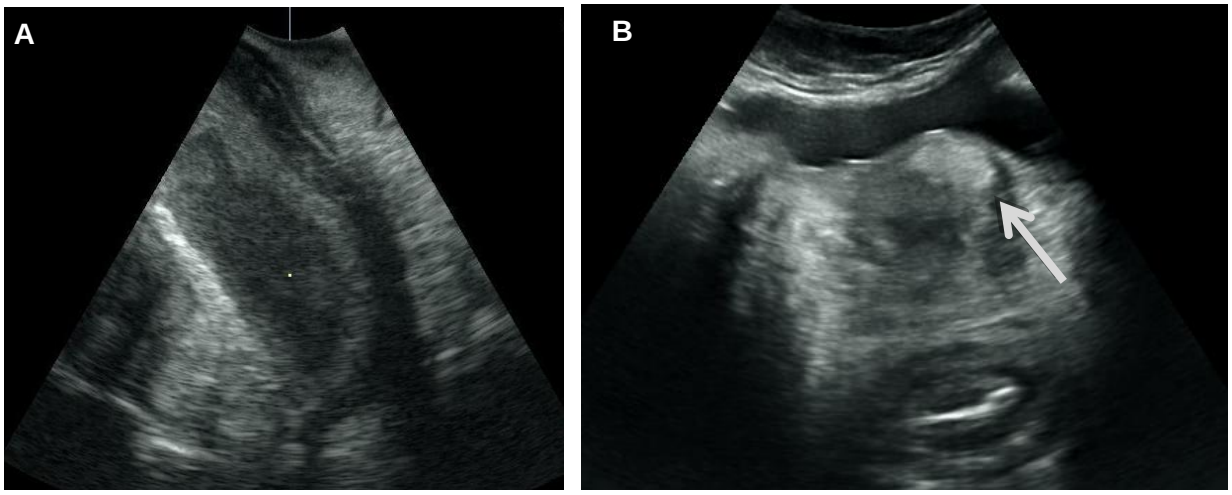

Fig 18 (A) Transrectal view of the uterus filled with echogenic fluid occupying the lumen with a volume of 42.5 cm^3; (B) transabdominal transverse view of an enlarged cervix with a hyperechoic intramural mass (arrow) in a fluid filled uterus.

The solid mass displayed on Fig 18 B, is suspected to cause, in its full extension, the occlusion of the endocervical canal and subsequent haematometra (Fig 18 A).

Considering the ultrasonographic characteristics of the lesion shown on Fig 18 B, a hyperechoic deep intramural/subserous solid mass, with local tissue invasion, associated with haematometra, is highly suggestive of cervical malignancy, as some reported cervical tumours are hyperechoic and share these same features (Epstein et al., 2010).

However, diagnostic imaging of cervical malignancy with ultrasound is limited since there are frequent overlapping signs between malignant and benign lesions, especially during the early stages of cervical cancer (Rezvani & Shaaban, 2009).

Resorting to Doppler colour flow (not shown), increased vascularization of the cervix was observed, suggesting neovascularization, but peripheral vessels could not be reliably ascertained as neovascularized. Thus, the type of lesion remained unknown and further diagnostic tests were requested.

Differential diagnoses were: cervical malignancy, uterine and bladder neoplasias, cervical benign lesions (leiomyoma, lipoma, cervical polyp and endometriosis).

Cervical neoplasia in non-human primates has been scarcely reported if compared to the human female (Cooper & Gabrielson, 2007). Nevertheless, Wood, Borgerink, Register, Scott & Cline (2004) demonstrated prevalence up to 19% in cervical intraepithelial neoplasia in cynomolgus macaques.

Moreover, a squamous cell carcinoma of the vagina, cervix and uterus has been described in a gorilla with intermittent vaginal bleeding similar to this case (Stringer et al., 2010) contributing greatly to the primary suggested diagnosis of cervical malignant tumour.

On the other hand, cervical leiomyoma has also been described in great apes, namely in chimpanzees, and is a probable second diagnosis for this case (Brown et al., 2009).

The association of cervical neoplasia with Papiloma viruses has been reported for non-human primates (Wood et al., 2007), but not yet in great apes.

Jenny died in February 2012 in Pretoria Zoo at the age of 55, from heart failure due septicaemia and purulent abscessation in the liver, suspected to be caused by *Echinococcus* infestation, as dry protoscolices were found during hystopatological examination.

Unfortunately, histopathology was not performed on her reproductive tract.

Progressive, multiorgan involvement with *Echinococcus vogeli* causing secondary infertility was documented in gorillas (O'Grady, Esra, Yeager & Thomas, 1982) which remotely raises the hypothesis of the cervical lesion being also caused by echinococcosis (Dhaifalah, 2001).

3.4. Adnexal Sonopathology

Regarding adnexal lesions, this work describes cystic formations in the ovaries, complex ovarian masses, non-ovarian adnexal cysts and dilated fallopian tubes associated with pelvic masses.

3.4.1. Cystic formations in the ovaries

Several animals were found with cystic formations in the ovaries. In fact, it accounts for the majority of the pathological findings in this study.

However, the scanned ovarian cystic lesions had different ultrasonographic appearances (Fig 19). Most of the subjects with these lesions were orangutans (Karolin, Moni, Sekara and Tiba) and 3 were gorillas (Dufte, Liesel and Sakina). Most of orangutan subjects were asymptomatic, while in the gorillas' case some non-specific symptoms were present but rather associated with other lesions.

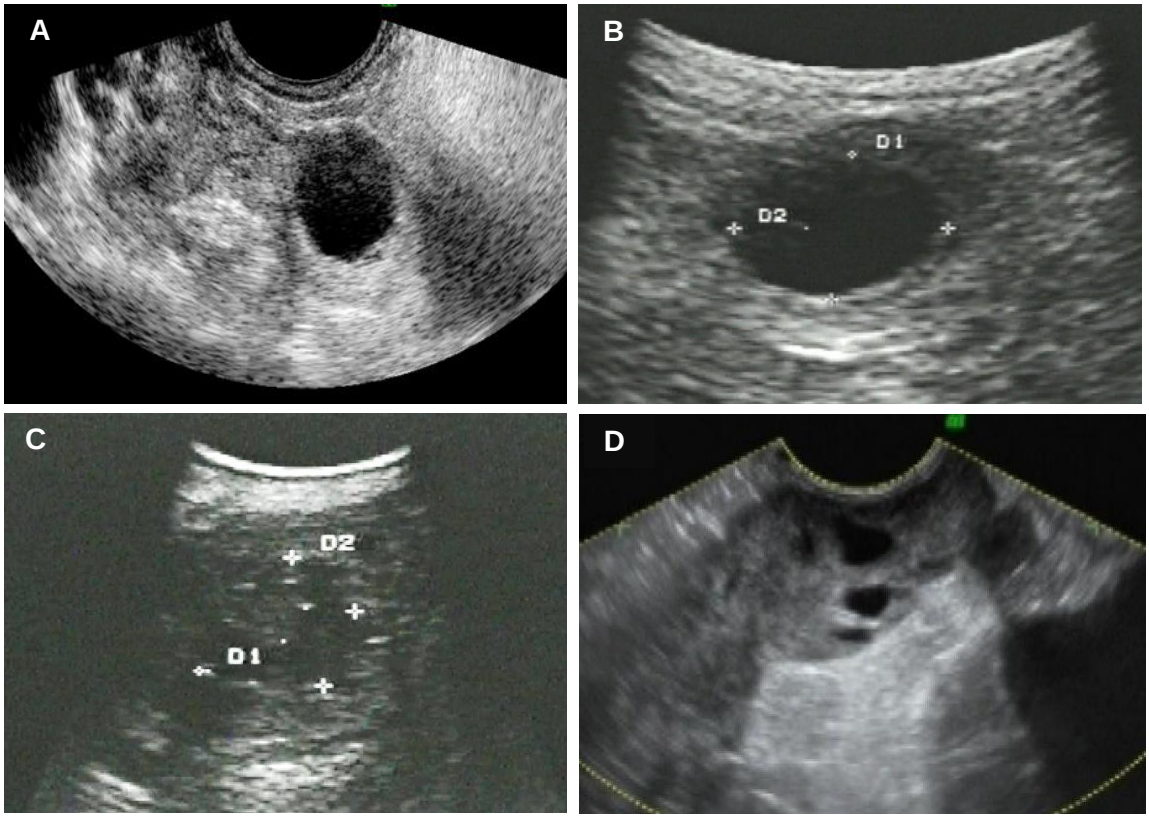

Fig 19. (A) Transrectal view of the ovaries of an orangutan, Tiba, with a large cystic structure (size in cm 2.62 x 3.27) and irregular walls; (B) transrectal view of the ovaries of orangutan, Karolin, with an oval shaped cystic structure with thin walls (size in cm 0.85 x 1.49); (C) transrectal view of the ovaries of gorilla, Dufte, with a cystic lesion displaying thick walls (cyst size in cm 2.08 x 1.61); (D) enlarged ovary (size in cm 4.84 x 2.36) with multiple cystic structures (>8 cysts up to 0.98 cm in size) in gorilla, Sakina.

An ovarian cyst is defined as a collection of fluid, surrounded by a visible wall, within an ovary, with a size larger than about a Graafian follicule (Cooper & Gabrielson, 2007).

Some ovarian cysts are functional in nature, i.e., develop during a normal menstrual cycle, being either follicular or luteal cysts.

Other ovarian cysts are non-functional, like a polycystic-appearing ovary (usually related to an endocrine disorder), chocolate cyst (endometrioma), haemorrhagic ovarian cyst, dermoid cyst, cystadenoma and borderline tumoural cysts (low malignant potential).

In non-human primates ovarian cysts are one of the most common findings and their relationship to impaired fertility is still uncertain (Roberts, 1986).

In women, small simple ovarian cysts are very likely benign lesions with malignancy rates of less than 1% (Levine et al., 2010). Large ovarian cysts with solid elements with central flow on colour Doppler US raise suspicion for malignancy (Levine et al., 2010).

Even in the largely studied human imaging, it can be challenging to distinguish and assess all these types of ovarian cysts by means of ultrasonography, however, there are some guidelines based on the recognition of characteristic morphologic patterns, hormonal levels, cyst persistency and existence of symptoms (Jaffe et al., 1994; Levine et al., 2010).

Thus, thin-walled, unilocular, round or elliptical anechoic structures within the ovary, up to 5 cm of diameter, that possess no flow on colour Doppler US and no septations or internal echoes, like the majority of the observed ovarian cysts in this study (Fig 19 A, B) were suggested to be functional follicular cysts. Supporting this diagnosis was the fact that most subjects were symptomless, exhibiting unexplained unwanted impaired fertility only in Conny's case.

In two orangutans, Karolin and Moni, the diagnosis of follicular cyst was indirectly confirmed, due to subsequent examinations performed 6 months apart that showed no signs of persistent of the ovarian cysts. They had been previously submitted to progestogen-only subdermal contraceptive implants (Norplant I and II – Jadelle®), which in women are frequently linked with functional ovarian cysts (Hidalgo et al., 2006).

According to Bolton, Masters, Milham & Lea's (2012) studies, contraceptive use was not a significant predictor of reproductive dysfunction for captive great apes. The suggested diagnosis of simple functional cysts in the contracepted animals, Karolin and Moni, seemed in accordance with Bolton's et al. (2012) finding, as they did not present a real disease.

On Fig 19 C, the displayed cyst had thicker walls, internal echogenicity and possessed peripheral flow on Colour Doppler imaging similar to a "ring of fire", which was suggested to be a luteal cyst (larger than a corpus luteum). Since Dufte had a concomitant pelvic infection, the observed ovarian cyst could represent a case of oophoritis, but it is not clear.

Fig 19 D shows a large ovary with multiple cysts on the periphery and increased echogenicity of the stroma. This is the typical ultrasonographic image of a polycystic ovary, and it was visualized in 2 subjects, Sakina and Sekara. In humans, polycystic ovarian morphology on

US can be caused by hyperprolactinemia, hyperandrogenism (polycystic ovarian syndrome, PCOS), hypothyroidism, hypothalamic dysfunction or it can be idiopathic. In general, if a hormone disorder is present, anovulation occurs and impaired fertility is the expected consequence (Gadir et al., 1992). Sakina's blood results showed increased prolactin levels and low LH and estradiol, indicating no recent ovulation and no follicular activity, which highly suggested that the cystic structures observed in her ovaries were not follicles but cysts, and thus, that she had a polycystic ovary. She was given Cabergolin orally (3 mg per week) to reduce her prolactinemia, but it did not show the desired effect as she continued to present no sexual activity and the lesions continued to grow.

The possibility of Sakina having PCOS, was not explored, since complete diagnostic data was not disclosed in her case (e.g. serum androgen levels) and typical symptoms, like obesity and virilisation (Abbott, Colman, Kemnitz, Eisner & Dumesic, 2002) would be difficult to visualize or associate with PCOS, in a great ape.

The *post-mortem* histopathological report after her death, the existence of hemo-purulent material and *E.coli* bacterial culture from the cysts at the site of the ovary and in the uterus, suggested that her peritonitis was septic, and thus it could not be simply caused by the rupture of large ovarian cysts (chemical peritonitis). It is hypothesized that she developed a pelvic infection affecting her reproductive organs (including her polycystic ovary and adenomyotic uterus) either by the hematogenic via, transperitoneal via from adhesions with contiguous organs (GIT) or by ascending pelvic inflammatory disease (PID) - see 3.4.4. pelvic masses discussion.

3.4.2. Complex ovarian masses

Two subjects demonstrated ovarian masses with solid and cystic components, Amani and Liesel (Fig 20). Both female gorillas had non-specific symptoms, only lack of sexual behaviour and weight-loss.

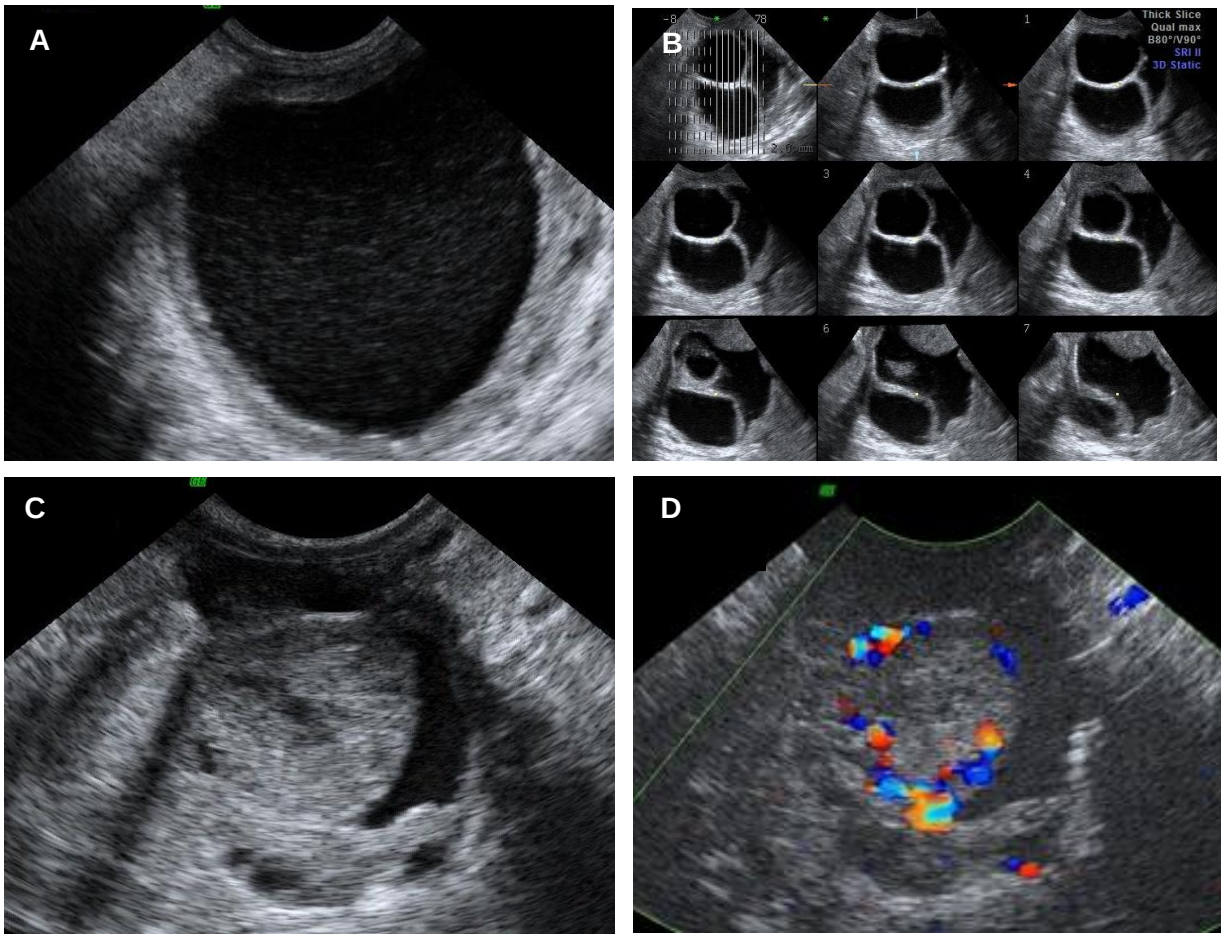

Fig 20 (A) Transrectal US image displaying a mixed mass with a thick hyperechoic wall with echoic foci surrounding a cystic cavity filled with low-level echoes, in a gorilla, Amani; (B) tomographic ultrasound imaging of the septations within the mass displayed in A; (C) transrectal view of the ovaries of gorilla, Liesel, with a large solid/cystic mass (size in cm 1.58 x 2.24) with irregular walls and septations (size in cm 0.86 x 3.33); (D) colour Doppler view of the mass in C, displaying a ring of vessels around it and on the septations.

Amani's ovarian mass ultrasound appearance is indicative of an Endometrioma (or Chocolate cyst) due to the existence of low level echoes evenly distributed throughout the cyst and to its septations (Fig 20 A, B). Also supportive of this diagnosis is the fact that in non-human primates, ovarian masses are most often endometriosis, not neoplasia (Cline et al., 2008). Therefore, ovarian tumours were kept as secondary diagnosis for Amani's case.

On the other hand, regarding Liesel's mass (Fig 20 B, C) with apparent malignancy signs, such as thick nodule/septations and flow on colour Doppler US, the primary diagnosis was Ovarian tumour; and a corpus luteum cyst was considered as secondary diagnosis, requiring further assessments to distinguish the two.

Ovarian neoplasia is sporadically reported in great apes. According to Brown et al. (2009) the ovarian tumours already described in chimpanzees are: fibrothecoma, granulosa cell tumour, teratoma, brenner tumour, Sertoli-Leydig cell tumour. In gorillas, reports have described an

ovarian endometrioid adenocarcinoma (Huntress, Loskutoff & Raphael, 1988) and an ovarian choriocarcinoma (Cook et al., 1995). A retrospective survey of medical and pathology records of great apes at the National Zoological Park only referred the occurrence of a malignant granulosa cell tumour in an orangutan but no reproductive neoplasia in the gorilla (Munson & Montali, 1990). Due to the scarce reports on this type of pathology in great apes, prevalence cannot be deducted. However, a prevalence study of ovarian tumours in baboons stated that they represent 7–15% of all neoplasms, of which approximately only 20% turn out to be malignant (Moore, Hubbard, Leland, Dunn & Best, 2003).

In this study there was no definitive diagnosis for endometriosis, although, according to the literature, it has been described in great apes (Doré & Lagacé, 1985; Munson & Montali, 1990). The disease prevalence among non-human primates necropsied between 1981 and 2001 in the same colony was 31.4% and it increased with age (Zondervan et al., 2004).

Endometriosis has been often associated with impaired fertility (Doré & Lagacé, 1985; Roberts, 1986).

Amani was considered for reversible infertility management and she was given a GnRH vaccine as an attempt to treat her lesions (endometriosis or ovarian cancer) (Nelson, 2007). Nevertheless, she died and a histopathology was performed. Results are still pending.

Ovarian tumours have better prognosis in non-human primates (NHP) than in humans, because of a marked difference in cell origin and malignancy proportion of these lesions in both species. Whereas in humans, the most common ovarian tumour (65% of ovarian neoplasms) is the surface epithelial cell type (of which 90% are malignant), in the NHP, 38% of ovarian tumours derive from sex cord-stroma (of which only 17% are malignant), 25% from germ cells (10% malignant), 23% from surface epithelium (39% malignant), and 14% from other tissues (91% malignant) (Cooper & Gabrielson, 2007).

As for Liesel, the first IZW ultrasound examination took place on July, 2008 and the result was unclear since no particular characteristics were seen on the ultrasound exam to distinguish ovarian tumour or irregular corpus luteum. A second US examination happened on 23 September, 2008 and this time an ovarian tumour was diagnosed (though CL cysts can take up to 3 months to disappear). The tumour diagnosis was supported by the extreme elevation of some human tumour markers in the blood (CA125, cancer antigen) and hence surgery for removal was scheduled. The surgery took place on January, 2009 with the involvement of a human surgeon. He did not find a tumour, but noticed an irregular corpus luteum cyst. Explanation for the high CA125 levels can be attributed to her cystic myometrial lesion thought to be a leiomyoma or adenomyosis (Moyle et al., 2010).

She was supplemented with L-Thyroxin and Bromocriptin (dose undisclosed) in an effort to treat the underlining cause of her ovarian lesion (hormone disorder, most likely hypothyroidism) and to resolve anovulation keeping pregnancy possible. She has not produced offspring since 2000, but she is alive and well.

3.4.3 Non-ovarian adnexal cystic structures

Cystic masses in the adnexal region were encountered in 4 subjects: gorillas, Kolo (14), Dian (21) and Zaire (32), and a chimpanzee, Lillie (26) (Fig 21). Dian was examined 14 days after an episode of severe bleeding from the anogenital region. The bleeding ceased in the same day without intervention and she returned to her normal overall condition. Zaire and Lillie had only unspecific symptoms, namely impaired fertility and depression. Kolo was being scanned to check for pregnancy, but it was negative.

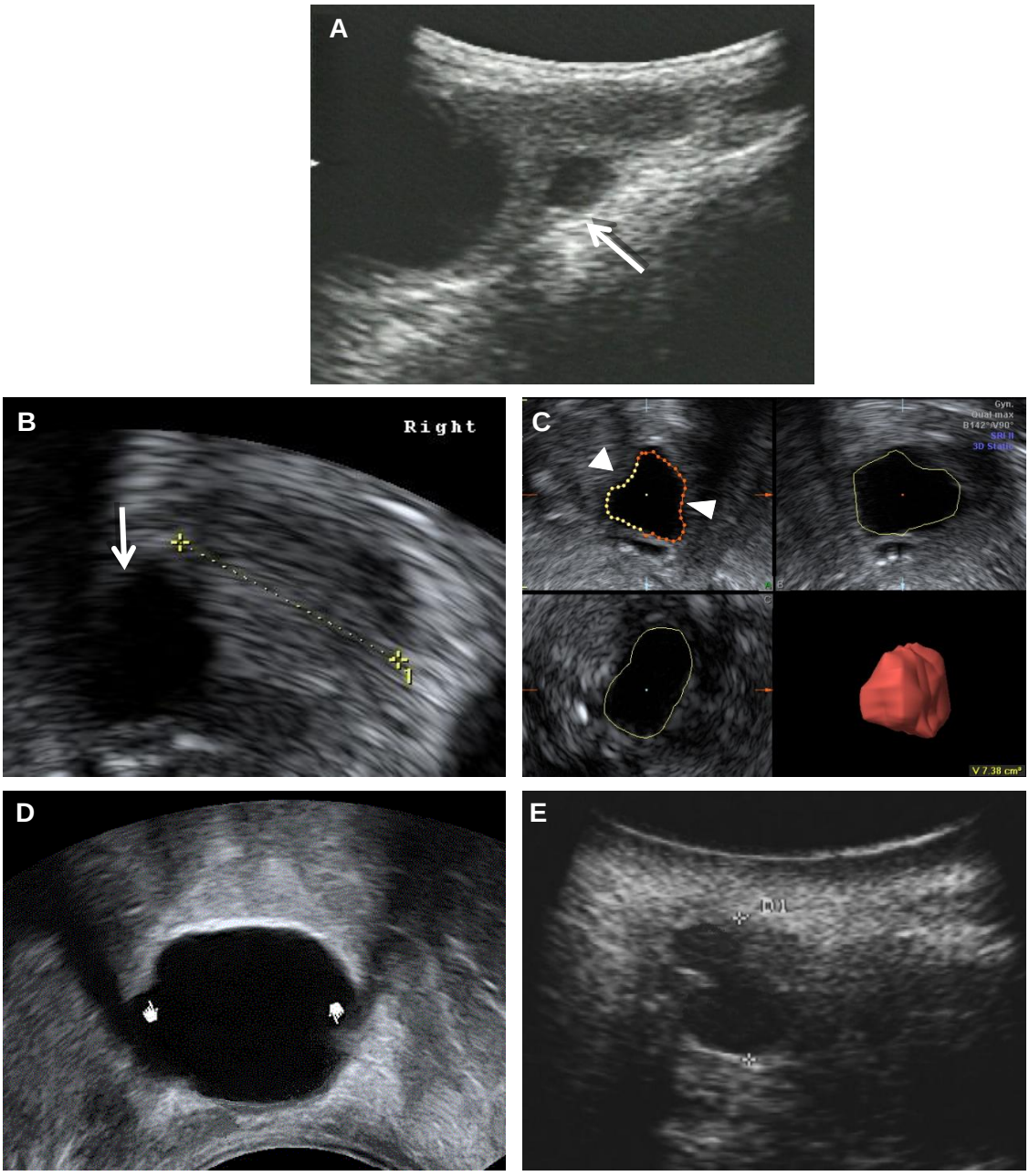

Fig 21. (A) Transrectal view of a small round cystic structure (arrow - 0.61 cm of diameter) near the ovary of gorilla, Kolo; (B) transvaginal view of an adnexal cyst (arrow) in the amenities of a very inactive ovary (crosshairs) of a gorilla, Zaire; (C) tubular conformation, waist sign (arrow heads) of the cyst in B visible after TVUS 3D calculation); (D) sausage shaped cyst (size in cm 2.81 x 3.58) with projections (cursors) located in the adnexa of gorilla, Dian; (E) c-shaped cyst (crosshairs) near the ovary of a chimpanzee, Lillie.

Accurate distinction between adnexal cysts can be difficult, but it is possible to identify different adnexal lesions on the basis of their ultrasound characteristics, as presented in Table 8.

Table 8. Distinguishable US characteristics of paraovarian cysts, hydrosalpinges and peritoneal inclusion cysts in women (Savelli et al., 2006).

	Paraovarian cysts	**Hydrosalpinges**	**Peritoneal inclusion cysts**
Ipsilateral ovary	Yes	Yes	Yes
Morphology	Ovoid	Tubular	Irregular
Proper wall	Yes	Yes	No
Papillae	Yes	No	Yes
'Beads-on-a-string'	No	Yes	No
Complete septa	Rare	No	Frequent
Incomplete septa	No	Frequent	Rare
'Flapping sail sign'	No	No	Yes
'Split sign'	Yes	Yes	No

Thus, based on Savelli's et al. (2006) retrospective studies in women, the round cyst displayed in Fig 21 A was suggested to be a paraovarian/paratubal cyst.

Conversely, the variable shaped cysts in Fig 21 B, C and D are most likely not paraovarian cysts, since their shape is not regularly ovoid. In addition, they don't possess characteristics of peritoneal inclusion cysts, and neither yolk sac nor a fetal pole typical of ectopic pregnancy. Moreover, the recognition of the ipsilateral ovary in 21 B and D and the visualization of a clear separate (non-ovarian) location excluded ovarian cysts.

Therefore, they were suggested to be "tubal cysts", i.e. hydrosalpinx (distal oviductal blockage).

The recognition of clear fluid within a cyst of tubular configuration contributed to this diagnosis, but tubular obstructions can have several appearances: a thin or thick-walled c-shaped anechoic tubular structure, like in Lillie's case (Fig 21 E) or, alternatively, as is Dian's and Zaire's case, it can appear as sausage shaped cyst (Fig 21 C, D) separate and distinct from the uterus and ovary (Patel, Acord & Young, 2006).

On Fig 21 C, it was possible to visualize indentations on both sides of the cyst (arrow heads), called the "waist-sign", and on Fig 21 D, small round projections (cursors), called "beads-on-a-string" were present on the wall. Both these findings are highly predictive of hydrosalpinx according to Brown, Dudiak & Laing (2010).

The typical symptoms of subjects with hydrosalpinx are pelvic pain or reduced fertility, but often they are asymptomatic. The most common cause for hydrosalpinx oviductal blockage in women is adhesions from prior episodes of pelvic inflammatory disease. Other causes include endometriosis, tubal malignancy and ectopic pregnancy (Kim et al., 2009).

Dian's sudden menorrhagia episode was suspected to have been an abortion: 14 days later she was examined and her hCG levels (hCG > 400 IU/L serum) were consistent with this suspicion – i.e within 14 days of an abortion the body has not yet been able to lower the pregnancy high hCG levels to the normal concentration of 5 IU/L (in humans) (Barnhart et al., 2004) and near zero in non-pregnant chimpanzees (Shimizu et al., 2003). A theory was elaborated suggesting that she had a spontaneous tubal missed abortion, where the tubal mole was partially absorbed/partially liquefied, giving rise to a small and often symptomless hematosalpinx, which eventually cleared (Verghese, 2006). Dian became pregnant approximately 1 month after being suggested to have a unilateral hydrosalpinx/hematosalpinx and no treatment was applied.

Pelvic inflammatory disease has been suggested previously in great apes (Munson & Montali, 1990) and studies in secondary impaired fertility in gorillas by O'Grady et al. (1982) link hydrosalpinx with an inflammatory process, infectious or otherwise. So, though speculative, it is suggested that Zaire and Lillie developed hydrosalpinx due to a previously undiagnosed subclinical PID, in which bacteria lodged in the Fallopian tubes and caused the destruction of tubal cells, leading to scar tissue formation in the distal end and to the obstruction of the pathway, seen as trapped fluid accumulation.

Paraovarian cysts are considered incidental findings in macaques, and they were found to have no effect on ovarian activity (Marr-Belvin et al., 2010).

Hydrosalpinx has been described in several gorilla candidates for assisted reproduction techniques, since detection of this lesion usually indicates lack of tubal patency due to blockage of an oviduct. Impaired fertility is associated with hydrosalpinx especially if bilateral (Lanzendorf et al., 1992; Hatasaka et al.,1997; Loskutoff et al., 2003).

3.4.4. Dilated fallopian tubes associated with a pelvic mass

This study presented 2 cases involving pelvic masses and dilated fallopian tubes with hypoechogenic material: Rawit, a 25 year old orangutan, and Dufte, a 21 year old gorilla, both presenting signs of acute pelvic pain (Fig 22).

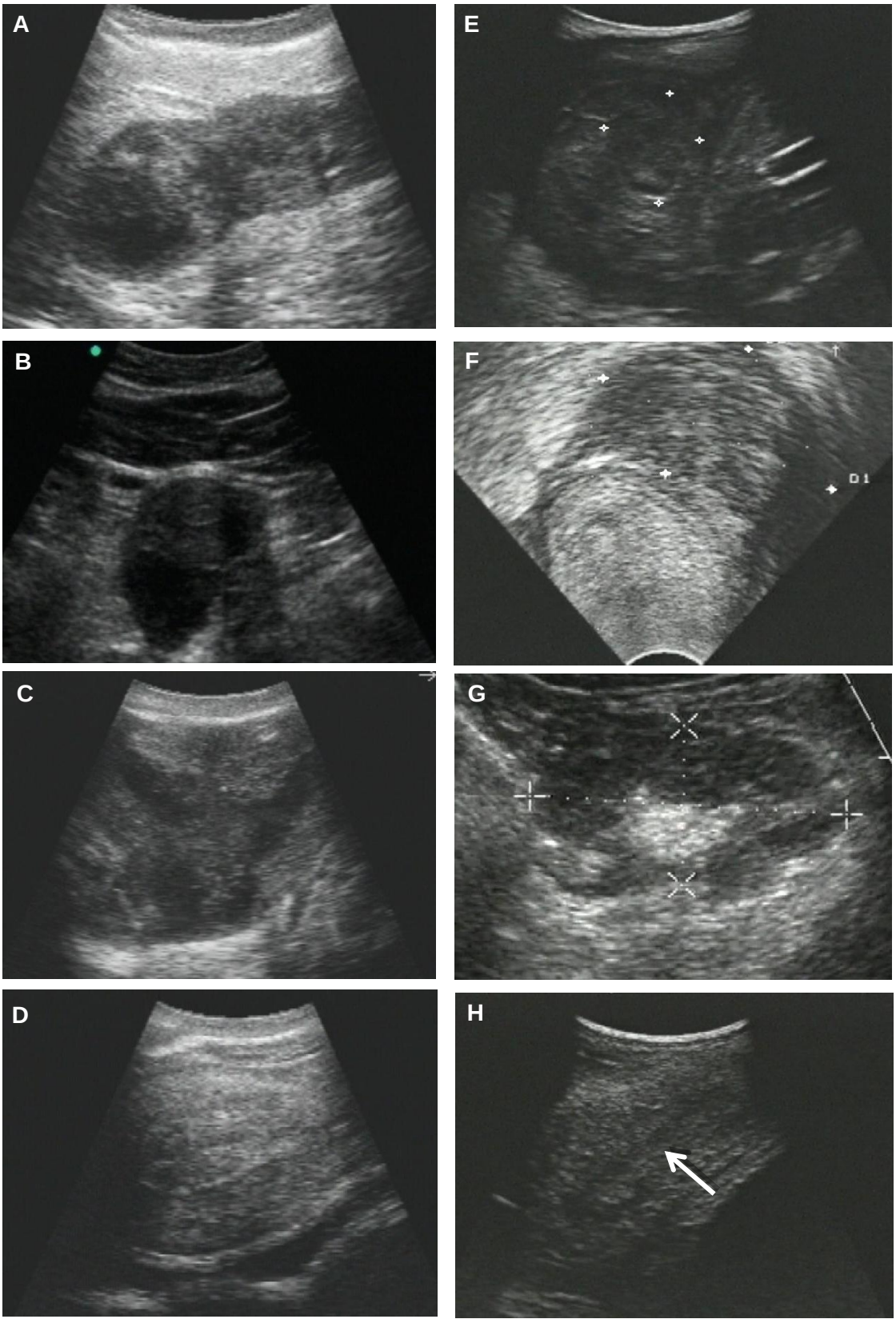

Fig 22. 1st column, Rawit: (A) dilated fallopian tube viewed transrectally; (B) transabdominal view of an undefined anechoic mass between the GIT and the adnexa; (C) pelvic abscess dorsolateral to the uterus viewed transrectally; (D) peritonitis in the douglas pouch; 2nd column, Dufte: (E) mass lateral to the uterus, apparently encarcerating the right ovary (crosshairs), viewed during transrectal US and flushing; (F) pelvic mass adjacent to the uterus viewed transvaginally; (G) transabdominal view of an abdomino-pelvic mass (size in cm 8.2 x 3.9); (H) thickened edematous endometrium (arrow).

Based mainly on ultrasonographic appearance the combined analysis of the US images displayed in Fig 22 lead to the suggested diagnoses of: tubo-ovarian abscess (TOA), GIT abscess, abdominopelvic neoplasm and pelvic endometriosis.

Surgery was performed on both subjects and a pelvic abscess involving the GIT and the reproductive tract was confirmed. The aetiology of the pelvic mass required further interpretation of the performed tests, as abscess origin is frequently difficult to ascertain due to severe adhesion formation (Mylniczenko, 2003).

Since abscesses are usually caused by an infection, endometriosis would be apparently excluded, as it is usually a disease related to sterile peritonitis and adhesions (Doré & Lagacé, 1985). Nonetheless, 2 reported cases in baboons maintained the aggressive endometriosis hypothesis reasonable: one case of exudative bacterial peritonitis related to external endometriosis (DaRif, Parker & Schoeb, 1984) and endometriosis with ileocaecal involvement and regional lymph-node enlargement (Barrier et al., 2007).

In Rawit's case, initially, the symptoms lead the Zoo staff to suspect she was pregnant. According to Zoo keepers it is not uncommon when animals are quiet and lose appetite just before birth, so they decided to wait for further developments, since a hasty investigation could jeopardize the possible baby. Unfortunately, in less than 3 days, her situation aggravated. By then, she was anaesthetized and an abdominal ultrasound was performed. Upon this exam, an abdomino-pelvic mass was found and an intestinal tumour was suspected (Fig 22 C).

Surgery was scheduled for the day after, when a complete ultrasound investigation was performed, but she died from endo-intoxication before the surgery could start. Histopathology was then included in the report. On her final ultrasound examination, together with the pelvic mass, exudative peritonitis was observed (Fig 22 D) and the adnexa were inflamed and filled with pus (pyosalpynx – Fig 22 A, B).

The acute deterioration of Rawit's condition pointed in the direction of a spreading infectious disease (with GIT or reproductive tract origin) and/or intestinal rupture (from infection, pelvic endometriosis, tumour or trauma).

The histopathology report allowed the preliminary dismissal of the differential diagnoses:

a) no tumoural cells were found in the intestine and no endometrial tissue was found outside the uterus;
b) there was no evidence of endometrial inflammation histopathologically, but the uterine serosa and outer myometrium were infiltrated with inflammatory cells, which led to the conclusion that the infection was not ascendant.

The definition of PID by the World Health Organization (WHO) is that of a clinical syndrome due to the ascent spread of vaginal and/or cervical microorganisms to the endometrium, Fallopian tubes, ovaries and adjacent structures. This definition excludes the cases where

the inflammation of the upper genital tract is caused by descendent infection from contiguous infected areas, such as the intestinal tract. Thus, the absence of endometritis left out PID by definition, but not a TOA (Aguirre et al., 2005).

Trauma to the abdomen with intestinal rupture was also a possible cause for the abscess formation, and spread to the reproductive tract, but there were no evidences of injuries in Rawit's body nor did the Zoo staff witness any fights or accidents.

Finally, after gathering all the information obtained from Rawit's exams and anamnesis, the hypothesis of acute diverticulitis/appendicitis with descendent infection to the reproductive tract (with eventual intestinal perforation), seemed to be the most plausible for her case.

According to Cox (1952) descending infection from the colon to the reproductive tract can occur by enterotubal fistula, transperitoneal spread, and through hematic or lymphatic spread. Supporting the theory of diverticulitis were 2 reported cases in orangutans: one with a ruptured intra-pelvic abscess spreading out into the reproductive tract (Pollock, Doyle, Tobin, Davison & Bainbridge, 2008) and another with ruptured diverticulitis and fatal peritonitis (Murray et al., 2000).

In Dufte's case, however, the endometrium appeared oedematous and irregular on ultrasound examination (Fig 22 H – supposed endometritis due to the presence of uterine fluid, which is not clearly visible) and raised suspicion for PID (ascendant reproductive tract infection). Also, the location of the pelvic mass and the supposed incarceration of the ipsilateral ovary (Fig 22 E) were consistent with the description of a TOA (complicaded pyosalpinx -Fig 22 F) and its most common cause is PID (Beigi & Wiesenfeld, 2003). The PID could have spread to the adjacent structures (bowel, pelvic peritoneum) causing bowel inflammation as seen in Fig 22 G, where the abscess possesses a target pattern, typical of thickened bowel loops (Fried et al., 1996).

Additionally, Dufte was found to have a hepatic mass on ultrasound (not shown), possibly complementing the primary diagnosis. PID with intestinal and liver involvement, also called the Fitz-Hugh–Curtis syndrome, is reported in humans (Sam, Jacobs & Birnbaum, 2002) and in non-human primates by repeated inoculated infections (Patton, Kuo, Wang & Halbert, 1987). In great apes, PID has been suspected but not confirmed (Munson & Montali, 1990).

Therefore, descendant infection from the GIT infecting the reproductive tract, as described for Rawit and endometriosis with ileocecal involvement were strongly considered secondary diagnoses.

Dufte's exploratory laparotomy was performed in 1995, and diffuse peritonitis as a result of a ruptured intrapelvic abscess associated with large bowel adhesions was detected. The abdomen was flushed, necrotic debris and abscesses removed, but hysterectomy was not performed. This surgical procedure proved successful together with strong antibiotherapy as Dufte survived a few more years. However in 2001, despite the surgery, Dufte died suddenly of an apparent recurrence of the same disease, with pyometra, peritonitis and intestinal

perforation, before anything could be done by the local veterinarians. A histopathological report was not provided.

In conclusion, as mentioned previously in 3.4.1 as an explanation for Sakina's death, intra-abdominal abscesses are an important problem in captive gorillas (Mylnyczenko, 2003). This study described a similar occurrence in an obese orangutan female with impaired fertility. Rawit had a history of 2 still-births (1997, 1999) and no live conception up until her death. Dufte, on the other hand, had 2 live births until 1992.

3.5. Male reproductive tract sonopathology

In the males of this study only intra-scrotal lesions were found, namely testicular masses.

3.5.1. Testicular masses

Out of 7 male great apes examined, 3 had testicular masses, Jitu, Bobby and Kwanza, who were 25, 23 and 22 years old, respectively, at the time of their examinations. These masses had substantially different ultrasonographic appearances (Fig 23).

All male subjects were scanned as part of fertility evaluations, so no other symptoms were present, besides impaired fertility (low quality semen, low testosterone levels).

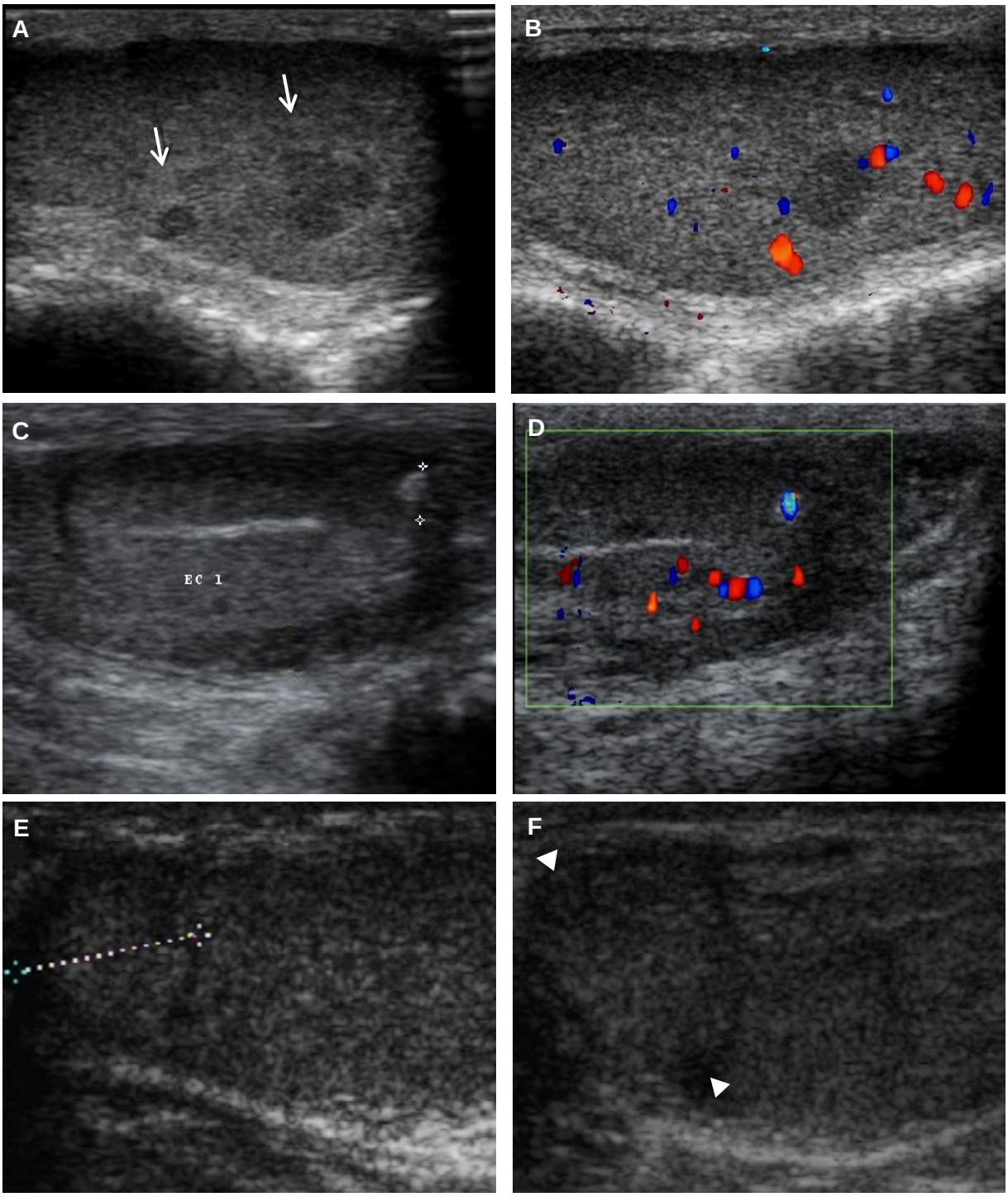

Fig 23. (A) Hypoechoic nodules (arrows) in Jitu's testicular parenchyma; (B) Doppler image showing vessels surrounding the lesions in Jitu's testis; (C) hyperechoic foci (crosshairs) in the testicular parenchyma of Bobby, and epididymis corpus; (D) colour Doppler image showing blood flow directly upon Bobby's parenchymal lesion; (E) large circumscribed mass (crosshairs) on Kwanza's right testicle measurering 1.06 cm; (F) Kwanza's testicular mass with slight heterogenous echotexture (arrow heads).

Although the sonographic appearance of each subject's lesion was different, they all had intra-testicular lesions with some characteristics in common with tumours:

- There were no other scrotal findings, such as enlargement and hypervascularity of the entire epididymis and testis, reactive hydrocele or pyocele and scrotal wall edema, which would support a diagnosis of inflammation and differentiate from tumours (Kim et al., 2007).
- On Bobby and Kwanza's lesions (Fig 23 C and E) the presence of a solitary intratesticular solid mass was highly suspicious for malignancy (Kim et al., 2007).
- On Kwanza's testicular mass (Fig 23 E and F) the echotexture was slightly inhomogenous, mimicking typical tumoural lesions (Dogra et al., 2003).
- The Doppler colour flow image of the testis was altered in Bobby's and Jitu's US examinations: by increased vascularity peripheral to the lesion (Fig 23 B) or directly upon the lesion (Fig 23 D) common in malignant lesions (Dogra et al., 2003).

These findings cannot confirm the malignancy of the encountered lesions, but they lead to tumour suspicion. That being said, with tumour suspicion more diagnostic tests are required, as there are a variety of benign intra-testicular processes, such as haematoma, orchitis, abscess, infarction, and granuloma that mimic testicular malignancy, and must therefore be considered in the differential diagnosis and investigated (Hamm, 1997).

Serum levels of androgens, magnetic resonance imaging (MRI) or a biopsy should be performed to exclude or confirm the malignancy. If the lesion turns out to be benign, then an organ sparing surgery can be feasible. But, if malignancy is confirmed, most likely, the prognosis is poor and the most common treatment is unilateral or bilateral orchiectomy to prevent further complications. (Dogra et al., 2003).

Jitu was hemi-castrated in 2008, after being ultrasonographically diagnosed with a testicular tumour. Histopathology was performed on his testis and a Leydig (interstitial) cell tumour, tubular atrophy and interstitial cell hyperplasia were detected.

These tumours have been reported in small numbers in great apes (Jones, Dixson & Wadsworth, 1980; Karesh, Burton, Russell & Burns, 1988) and the literature in non-human primates and other animals suggests that these tumours are more likely to be benign than malignant (Karesh et al., 1988). Nevertheless, a malignant Leydig cell tumour has been described in a non-human primate (Brack, 1988).

In Jitu's case there were several discrete presumed tumour nodules in the parenchyma as well as occasional intravascular nests of similar altered cells in the tunica, which could be a sign of an invasive lesion, but a definitive conclusion could not be made.

In sum, Jitu's ability to produce offspring with his contralateral testis is unclear, as is the cause of the encountered tubular atrophy and interstitial cell hyperplasia in the affected testicle. Some articles demonstrate that these histological lesions are a common male gorilla problem, frequently found in captivity, and most likely unrelated to neoplasia (Dixson, Moore

& Holt, 1980; Foster & Rowley, 1982; Enomoto, Matsubayashi, Nakano, Fuji-Hanamoto & Kusonoki, 2004).

Bobby died in December 2008 and the histopathology report to his reproductive tract indicated that he had bilateral testicular degeneration and tubular atrophy. The author contacted with the veterinarians from London Zoo to obtain more information about his death and their initial response was that he died from an immune mediated haemolytic anaemia, which was thought to be caused by brucellosis. This theory has not been confirmed, as the exact pathogen causing the disease and the antibody reaction has not yet been identified. Testicular atrophy can develop after severe forms of orchitis and protracted courses, so a hypothesis could be made that the brucellosis infection caused orchitis and the testicular degeneration resulted from replacement of the parenchyma by scar tissue. However, the veterinarians at the London Zoo suspect that the positive test result for *Brucella* was a cross reaction. Thus, the cause for his lesions remains unknown, and it is unclear if it is related to the agent that caused the haemolytic anaemia.

Nevertheless, as mentioned previously, testicular atrophy, interstitial hyperplasia, degeneration and fibrosis of seminiferous tubules are commonly reported in male gorillas and a combination of factors, such as dietary deficiencies, psychological factors or increased temperatures as occurs in cryptorchidism, are proposed as cause (Dixson et al., 1980). Other suspected causes for these dysfunctions in the testicular cellular structure are hypogonadotropism, neoplasms, inflammatory or vascular diseases, drugs and genetic disorders (Spindler & Wildt, 2010).

Ya Kwanza, the first gorilla born from artificial insemination, had as a juvenile an undescended small right testicle. Orchiopexy was performed when he was about 4 years old. He managed to breed successfully for a period of time, but later, he began losing his libido. Artificial insemination attempts were made to continue with breeding, but he was found to have poor quality semen and was failing to impregnate the females in the group. Upon ultrasound examination, suspicion was raised for a testicular tumour on the right testis due to the ultrasonographic appearance of the lesion (solitary, solid, inhomogenous) and also due to his history of undescended testis, which in humans highly increases the risk of testicular tumour (Mathers, Sperling, Rübben & Roth, 2009). Therefore, an unilateral orchidectomy was performed in 2006, to reduce malignancy risk.

Histopathologically there was no evidence of malignancy. This finding was corroborated histochemically by the negative result of the enzyme immunoassay for placental alkaline phosphatase (PLAP) performed on the testis tissue, denoting low activity of the known tumour marker in the examined sample. Fibrosis was visible surrounding the tubules, and though there were signs of spermatogenesis, none of the tubules contained recognisable mature spermatozoa.

It is thought that the surgery as a juvenile to correct his cryptorchidism might have caused the early testicular atrophy.

Clomifene citrate (dose not provided) was given to Kwanza since March 2009 until Dec 2009 and improvement was noticed on his testosterone levels and sexual behaviour, but still no pregnancy was achieved. His contralateral testis fertility is thought to be hindered by the common male gorilla problem described earlier.

The causes and effects of the histological lesions commonly detected in the testes of male gorillas are yet to be understood. According to Enomoto et al. (2004), the degeneration of seminiferous tubes with absence of spermatogenic cells occurs, in different degrees, both in senescent infertile gorilla males and in adolescent males of proven fertility. This led to the speculation that perhaps spermatogenesis in the gorilla is quite vulnerable to certain conditions regardless of age. Thus, males of any age category can be affected and the extent of the damage may or may not impair fertility. Though captivity is thought to be a possible enabling factor for these lesions, free-ranging male gorillas dying of acute problems were also found to have inactive-looking seminiferous tubules and abundant interstitial cells (Hall-Craggs, 1962).

Regarding the abundance of interstitial (Leydig) cells, often interpreted as hyperplasia, Enomoto et al. (2004) suggest that this might not be a pathological acquired condition, but rather a physiological trait of male gorillas, thought to be related to the greater body size and amount of muscle and stroma of these species.

Interestingly, despite the abundance of Leydig cells in gorillas, Short (1984) reports that their peripheral blood androgens levels are similar to those in chimpanzees and orangutans. This could be explained by the fact that gorillas have overall smaller testes.

4. Descriptive Statistics

After analysing the US examinations individually, the results were combined to retrieve information on the following topics:

4.1. Incidence of lesions by age category

4.2. Compilation of lesion type by genus and age category

4.3. Relationship between lesion occurrence and offspring production

4.4. Subjects with lesions: comparison of US fertility prognosis with offspring production outcome.

4.1. Incidence of lesions by age category

An overview of Fig 24 demonstrated that the age category most affected with lesions of the reproductive tract was the aged group (4/4), followed by the adults (16/22) and adolescents (1 out of 3), which was, somewhat, expected. Nevertheless, the adult group was the most numerous age category of this study (22/29), compared to adolescents (3/29) and aged animals (4/29). This means that the IZW team was more often requested to assess adult animals, which shows that the zoos had more concerns with the group meant to be in its reproductive prime.

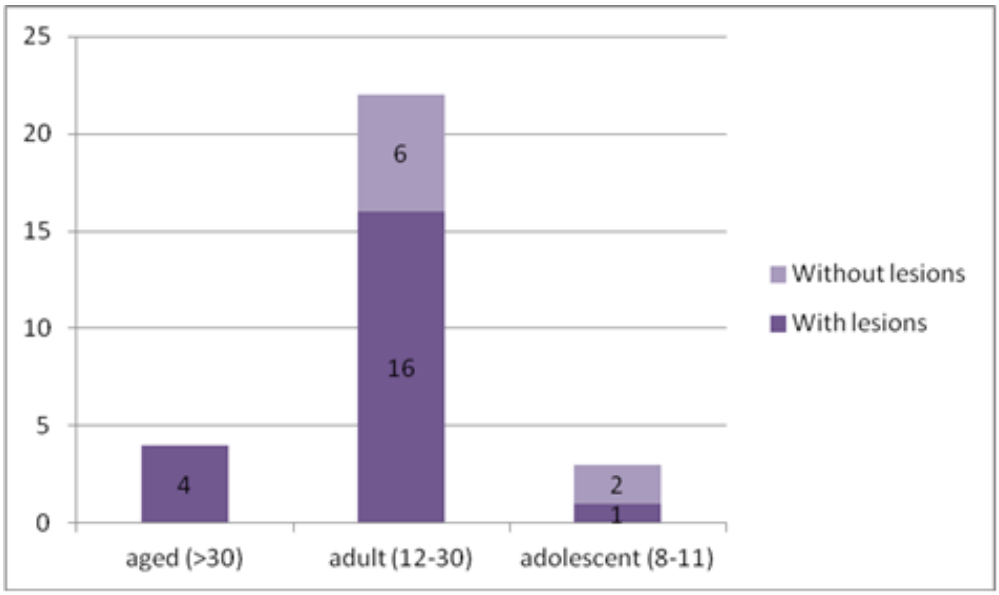

Fig 24. Ultrasonographic results displayed by age category.

Although the occurrence of reproductive lesions during reproductive age is not surprising, in this study there seems to be an abnormally high percentage of subjects with reproductive tract alterations before reaching 30 years of age (50% of the adult males and 81% of the adult females scanned). It is conceivable that captivity may influence the likelihood of disease development and compromising fertility.

It has been hypothesized that early depletion of ovarian follicles and early occurrence of reproductive lesions in great apes could be attributed to consequences of captivity, such as:

- shortened period of adolescent sterility (also called decreased age at first birth, shown in Table 2) – (Lathouwers & Elsacker, 2005).
- higher rates of miscarriage and stillbirths, followed by a faster return to cyclicity (also called interbirth interval, shown in Table 2) - noticeable especially in captive gorillas – (Bolton et al., 2012).

These captivity-related factors lead to an increased number of unproductive cycles during a life time and might predispose for the premature senescence of the reproductive tract (Atsalis & Videan, 2009).

A study done with baboons makes a similar assumption: more frequent unproductive cycling, consequent from captivity, might increase exposure to retrograde menstruation and to disease formation, since it was found that the prevalence of endometriosis increased with the duration of captivity (D'Hooghe, Bambra, de Jonge, Lauweryns & Konickx, 1996).

4.2. Compilation of lesion type by genus and age category

Considering the type of US lesion encountered amongst the great apes from the IZW database, Fig 25 was compiled. Due to the small population composing this study, more accurate conclusions require further assays. It should be remembered that some of the animals had more than one lesion, and thus, though 26 lesions were counted, only 21 animals had US alterations.

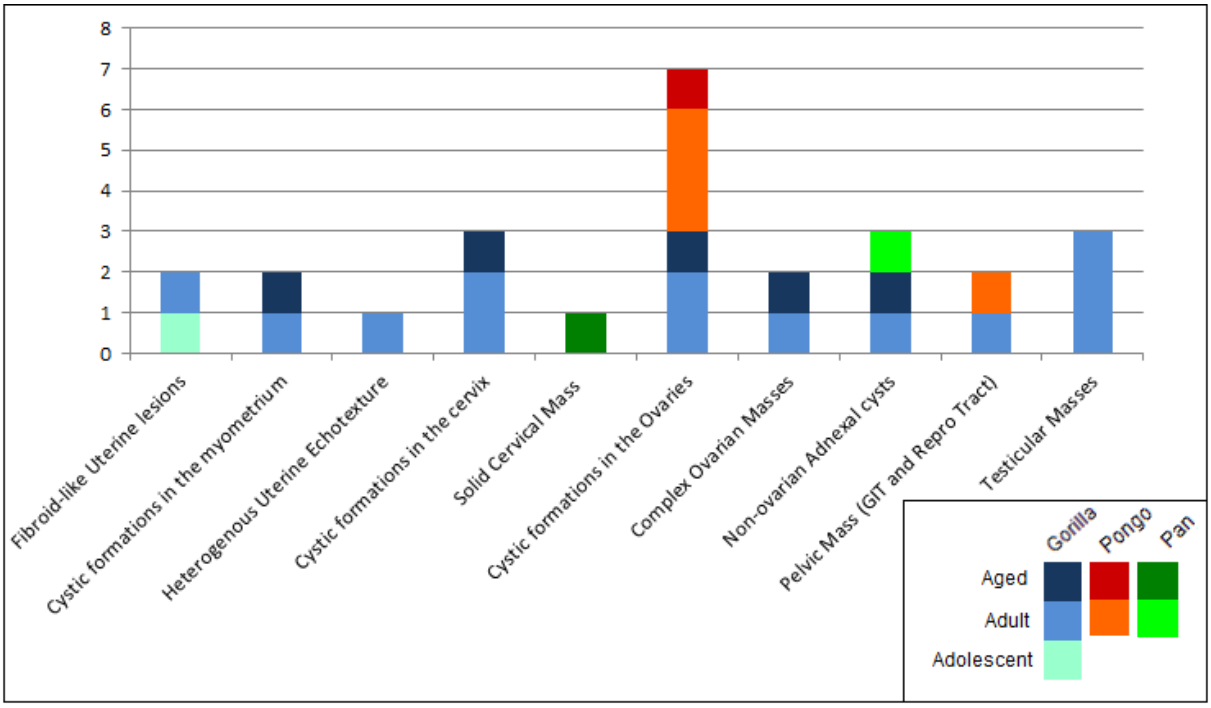

Fig 25. Compilation of the ultrasonographic lesions per species and age category (adolescent: 8-11 years old; adult: 12-30; aged > 30).

The most common lesion in this study was cystic formations in the ovaries, occurring predominantly in the genus *Pongo* (4/7). At least 2 of those were contracepted, to prevent

inbreeding because they were housed with their father and their line was over-represented. Since contraception data for the rest was not provided, the question is raised whether *Pongo* is particularly susceptible to implant progestagen contraception side-effects, or if a tendency for functional ovarian cysts is physiological in this genus.

Cystic formations in the cervix, non-ovarian adnexal cysts and testicular masses were the second most common lesions, occurring mostly in *Gorilla.*

One of the least common lesions was solid cervical mass. It was the only case of suspected malignancy in the cervix and it occurred in a member of the *Pan* genus, the closest genetically to *Homo* (Prüfer et al., 2012).

It is interesting to point out the occurrence of GIT abscesses associated with abscessed fallopian tubes (pelvic masses) in both a gorilla and an orang-utan. Since these two lesions are only occasionally associated in humans (Cox, 1952) and intra-abdominal abscess formation involving the reproductive tract has already been reported in gorillas and in orangutans (Mylniczenko, 2003; Pollock et al., 2008) it can be suggested that in great apes these lesions could be more frequently connected.

Testicular masses were present only in adult male gorillas. According to Enomoto et al. (2004), male gorillas frequently experience captive breeding problems related to testicular alterations; adolescents seemed less affected.

Furthermore, new information about offspring (from the updated species studbooks) after the IZW examination and the suggested diagnoses were added to Table 9.

Table 9. Characteristics of the scanned subjects, including the fertility prognosis and offspring production after the exam.

Species	Name*	Studbook no.	Sex (F/M)	Offspring prior to exam (Y/N)	Age at exam (Years)	Detection of us lesions (Y/N)	Sugested diagnosis	Fertility compromised	Offspring After exam (Y/N)
Gorilla *Gorilla g. gorilla*	Zaire	0557	F	Y	32	Y	Hydrosalpinx	Maybe	N
	Liesel	0649	F	Y	31	Y	Irregular CL cyst, uterine leiomyoma, nabothian cyst	Maybe	N
	Haloko	0393	F	Y	28	Y	Functional Ovarian cyst	Most likely not	N
	Kishka	0681	F	Y	28	Y	Leiomyoma, adenomyosis	Most likely yes	N
	Sakina†	0922	F	N	21	Y	Adenomyosis, pyoadenomyoma& policystic ovary	Most likely yes	N
	Amani†	0899	F	N	26	Y	Endometrioma	Most likely yes	N
	Jitu	0834	M	N	25	Y	Testicular tumour	Most likely yes	N
	Zsazsa	0941	F	Y	24	N	-	Most likely not	Y
	Bongo jr. (Bobby)†	1258	M	N	23	Y	Testicular tumour	Most likely yes	N
	Yakwanza	0855	M	Y	22	Y	Testicular tumour	Most likely yes	N
	Dufte†	0558	F	Y	21	Y	PID involving GIT/liver nabothian cysts & Ovarian cyst	Most likely yes	N
	Dian	1091	F	N	21	Y	Haematosalpynx	Maybe	Y
	Mike	1097	M	N	20	N	-	Most likely not	N
	Nyuki	1098	M	N	19	N	-	Most likely not	N
	Kolo	0936	F	N	14	Y	Paraovarian cyst	Most likely not	Y
	Bahasha	1328	F	N	12	N	-	Most likely not	N
	Mutasi	1316	F	N	8	Y	Intramural leiomyoma	Most likely not	Y
	Asante	893	F	N	19	N	-	Most likely not	N
Bonobo *Pan paniscus*	Zorba	168	M	Y	26	N	-	Most likely not	Y
	Ana Neema	?	F	Y	10	N	-	Most likely not	Y
Chimpanzee *Pan troglodytes*	Jenny†	?	F	Y	54	Y	Cervical tumour	Most likely yes	N
	Lillie	11888	F	N	26	Y	Hydrosalpinx	Maybe	N
	Phil	12883	M	N	11	N	-	Most likely not	N
Bornean orangutan *Pongo pygmaeus*	Rawit†	1778	F	N	25	Y	Diverticulitis involving reproductive tract (pyosalpinx)	Most likely yes	N
	Conny	2260	F	N	17	Y	Functional Ovarian cyst	Most likely not	N
	Karolin	1349	F	Y	26/27	Y	Functional Ovarian cyst	Most likely not	N
	Moni	1609	F	Y	22/23	Y	Functional Ovarian cyst	Most likely not	N
Sumatran orangutan *Pongo abelii*	Tiba	1499	F	Y	33	Y	Functional Ovarian cyst	Most likely not	N
	Sekara	2362	F	Y	19	Y	Polycystic ovary	Maybe	N

* † - Not alive in January 2013

4.3. Relationship between lesion occurrence and offspring production

Based on Table 9, a relationship between the occurrence of US lesions and offspring production is explored in Fig 26.

Of 29 scanned animals, 21 were detected to have reproductive tract lesions, and 8 were not. The large amount of suboptimal reproductive animals (with lesions, lack of offspring at some point or both) in this study is explained by the fact that the IZW group was usually called in by the Zoos to assess cases of reproductive symptoms or impaired fertility, rather than for routine scans, pregnancy checks, etc.

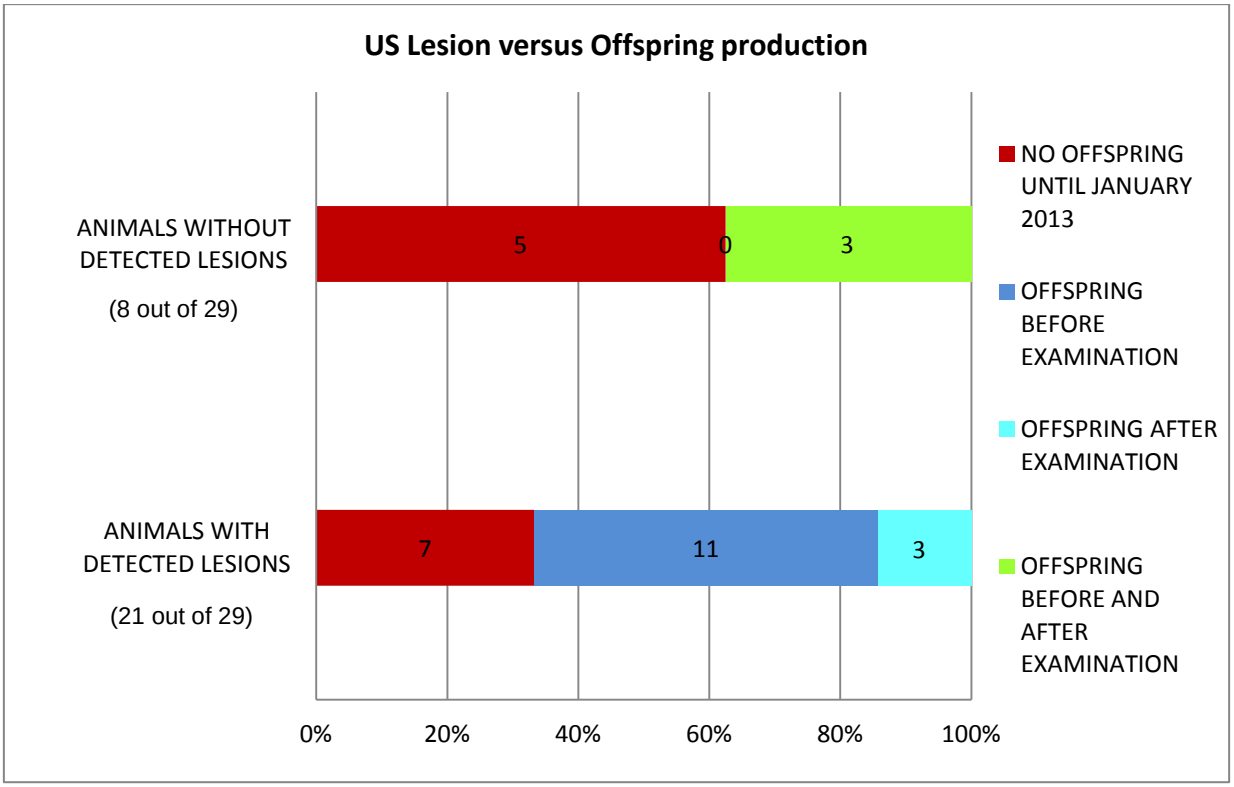

Fig 26. Percentage of animals with and without detected lesions upon ultrasound examination, discriminated by offspring production.

Fig 26 demonstrates that only 33% of the animals with detected lesions (7/21) were unable to produce offspring. The rest, 66% (14/21) had the ability to reproduce at some point, despite US alterations. A closer look on these 14 subjects shows that 11 (78%) of them produced offspring only before the examination, suggesting that their detected lesions were potentially acquired after the successful reproduction. The other 3 subjects (22%) with lesions had offspring only after the examination. These animals were 3 young gorilla females (8, 12 and 21 years old at the time of the examination) with suggested diagnoses of intramural uterine leiomyoma, paraovarian cyst and haematosalpinx following a tubal missed abortion, respectively. All 3 suggested diagnoses were compatible with further reproduction.

Regarding the group without lesions, an astounding 62 % (5/8) never produced any offspring. This could be explained by:

- (1) The US examination was insufficient to detect alterations (masked, hormonal, etc).
- (2) The reproductive problem is caused from psychological or social factors.
- (3) The fault of lack of offspring is on the reproductive partners they've been paired with.
- (4) The zoos prevented their reproduction, due to high risk of inbreeding within the group, or genetic disease.

For 2 of those 5 subjects, it was possible to determine the cause of the offspring absence:

- Bahasha's congenital vaginal septum but perfect integrity of the internal reproductive organs made her a good candidate for artificial insemination (AI). Until recently the reason for her nulliparity after AI was unclear. In late 2012, after introduction of a new breeding male, she is believed to be pregnant following frequent positive test results. This suggested that her earlier problem was included in item (3). Ultrasonographic confirmation of gestation is still pending.
- Phil fits in item (4), as he was castrated early in life, to prevent inbreeding in the group.

The other 3, have unknown causes for impaired fertility. In Asante's case, however, item (4) can be excluded and item (3) is not probable. She is thought to have psychosociologic stress and avoidance of sexual interactions, which puts her in item (2), though US insufficiency, item (1), is also plausible.

Lastly, 38% of animals that had no lesions (3/8) effectively produced offspring, and they did so before and after examinations, which indicated that no reproductive problem was indeed present.

4.4. Subjects with lesions: comparison of US fertility prognosis with offspring production outcome.

In an attempt to evaluate if offspring production, after detection of lesions, matched the fertility prognosis associated with the suggested diagnoses of this study, Fig 27 was composed based on data of Table 9, using the fertility terminology referred in 2.2.

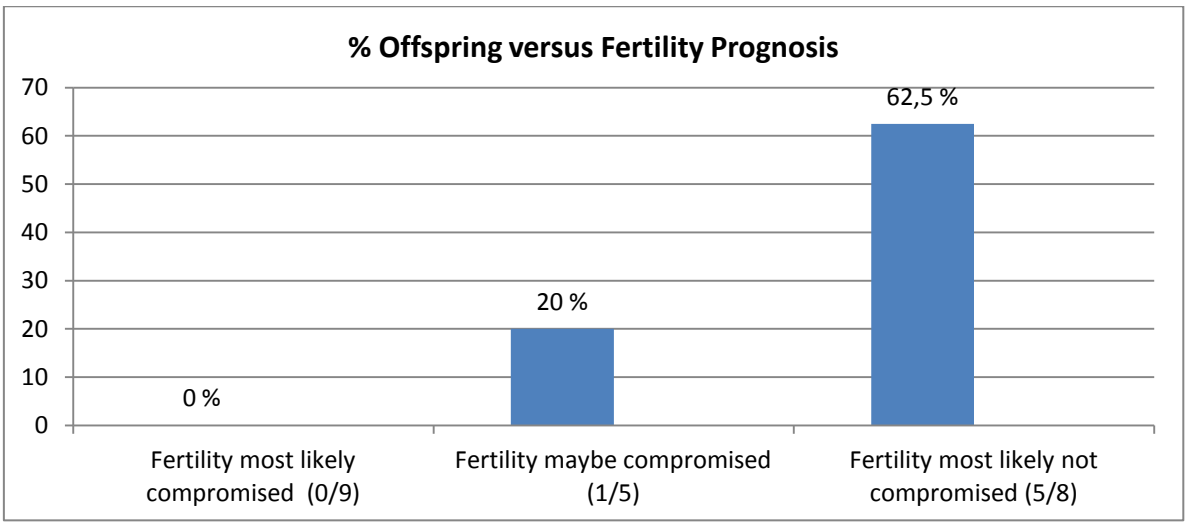

Fig 27. Percentage of animals with offspring (after lesion detection) in different fertility prognosis groups.

As can be seen in Fig 27, none of the subjects considered to have compromised fertility ended up producing offspring after the examination. Regarding the subjects whose diseases were thought to maybe compromise fertility, only 20% had offspring. Finally, of the great apes with apparently uncompromised fertility, nearly 63 % were able to produce offspring posterior to the exam.

These results lead to the assumption that the suggested diagnosis and fertility assessments provided by this study were relatively in accordance with the offspring production of the subjects. This is especially true in the cases when fertility was considered to be “most likely compromised”.

Thus, though offspring production could not be safely guaranteed in the apparently reproductively competent animals, ultrasound examinations of the reproductive tract of great apes have shown to be a useful tool to identify the reproductively incompetent animals, integrating an appropriate reproduction management program.

5. Final Considerations

This work presented a systematic study about reproductive ultrasound in great apes, describing its normal and abnormal appearance and attempting to assess the reproductive ability of the examined subjects. The results showed that detailed ultrasound examinations allow the detection of tissue alterations and the gathering of imaging signs sufficient for a suggested diagnosis. Unfortunately, in most of the cases, definitive diagnoses were not available, preventing the comparison with the presumptive ones. Nevertheless, through the suggested diagnoses it was possible to spot the animals most likely to require special management or assisted reproduction techniques, as shown by the descriptive statistics results. Thus, ultrasound revealed a high potential as reproductive management tool, particularly for fertility assessments. Continuous follow-up of the cases is recommended in order to confirm the suggested prognoses.

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